PONTÍFICIA UNIVERSIDADE CATÓLICA DO RIO GRANDE DO SUL
FACULDADE DE MEDICINA

LUCAS PORCELLO SCHILLING

INTEGRAÇÃO DE BIOMARCADORES DE NEUROIMAGEM NA DOENÇA DE ALZHEIMER: β-AMILOIDE, SUBSTÂNCIA BRANCA E METABOLISMO CEREBRAL

Porto Alegre
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Tese apresentada como requisito para obtenção do grau de Doutor pelo Programa de Pós-Graduação da Faculdade de Medicina da Pontifícia Universidade Católica do Rio Grande do Sul.

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Co-orientador: Prof. Dr. Pedro Rosa Neto

Co-orientador: Prof. Dr. Carlos Roberto de Mello Rieder

Porto Alegre
2016

Ficha Catalográfica

S334i Schilling, Lucas Porcello

Integração de biomarcadores de neuroimagem na Doença de Alzheimer - β-amilóide, substância branca e metabolismo cerebral / Lucas Porcello Schilling . – 2016.

138 f.

Tese (Doutorado) – Programa de Pós-Graduação em Medicina e Ciências da Saúde, PUCRS.

Orientador: Prof. Dr. André Luís Fernandes Palmini.
Co-orientador: Prof. Dr. Pedro Rosa-Neto.
Co-orientador: Prof. Dr. Carlos Roberto de Mello Rieder

1. Doença de Alzheimer. 2. Biomarcadores. 3. Tomografia por Emissão de Pósitrons. 4. Imagem de Tensor de Difusão. 5. Interação. I. Palmini, André Luís Fernandes. II. Rosa-Neto, Pedro. III. Título.

Elaborada pelo Sistema de Geração Automática de Ficha Catalográfica da PUCRS com os dados fornecidos pelo(a) autor(a).

Para minha esposa Martina

AGRADECIMENTOS

Ao Prof. André Palmini, orientador, amigo e inspiração desde a faculdade. Agradeço o apoio e confiança que me ajudaram a encontrar na neurologia minha vocação e contribuíram para a busca constante de conhecimento e qualificação.

Ao Prof. Pedro Rosa-Neto, orientador, amigo e modelo. Muito obrigado pelo carinho, paciência e dedicação no meu processo de formação como neurocientista.

Ao Prof. Carlos Roberto de Mello Rieder, pela atenção e apoio.

Ao grande mestre Prof. Jaderson Costa da Costa, pela confiança e incondicional apoio.

Ao Dr. Tharick Ali Pascoal, um grande amigo que a neurociência me trouxe. Minha gratidão por todo seu valoroso empenho e suporte, fundamentais nessa jornada.

Ao Dr. Eduardo Zimmer e aos demais pesquisadores e colaboradores do Translational Neuroimaging Laboratory da McGill, pela ajuda em toda a minha pesquisa com competência e seriedade.

Aos colegas e residentes do Serviço de Neurologia dos Hospital São Lucas da PUCRS pelo incentivo, auxílio e amizade.

Aos meus pais e exemplos, Luiz Fernando e Suzana, por tudo.

Aos meus irmãos Marcos e Martha, parceiros de sempre.

À minha avó Maria, que simboliza os valores e a força da família.

À minha segunda família, Guido, Cynara, Eduardo e Pedro, por todo apoio e carinho.

À minha esposa Martina, meu grande amor, maior incentivadora e companheira desta e de outras tantas jornadas.

“He who studies medicine without books sails an uncharted sea, but he who studies medicine without patients does not go to sea at all.”

Sir William Osler

RESUMO

A fisiopatologia da Doença de Alzheimer (DA) envolve diversos mecanismos patológicos, como acúmulo de β-amiloide e de emaranhados neurofibrilares, alterações na substância branca e neurodegeneração. Este trabalho consiste em um estudo transversal, cujo objetivo foi investigar os efeitos da integridade da substância branca e do depósito de β-amiloide como fatores determinantes do hipometabolismo cerebral no continuum da Doença de Alzheimer (DA).

Utilizando dados obtidos através do consórcio internacional *Alzheimer's Disease Neuroimaging Initiative (ADNI),* foram avaliados 96 indivíduos (27 sujeitos cognitivamente normais - CN, 49 sujeitos com comprometimento cognitivo leve – CCL, e 20 pacientes com DA) que realizaram um protocolo completo de neuroimagem com tomografia por emissão de pósitrons (PET) com [^{18}F]Fluordeoxiglicose ([^{18}F]FDG) e [^{18}F]Florbetapir e ressonância magnética nuclear (RMN) com a sequência de imagem de tensor de difusão (DTI).

Na primeira parte do estudo, no grupo DA foram identificadas áreas de redução da anisotropia fracionada (FA) no fascículo angular e fórnix. Dentre essas regiões, um voxel de interesse (VOI) foi bilateralmente selecionado no fascículo angular para as análises subsequentes. Após, examinamos a associação entre a FA no fascículo angular bilateral, o depósito de β-amiloide através do PET [^{18}F]Florbetapir Standardized Uptake Value Ratio (SUVR) nas regiões de interesse e a possível associação da interação entre ambos no hipometabolismo cerebral avaliado através de PET [^{18}F]FDG.

No grupo DA, a magnitude do hipometabolismo cerebral no corpo estriado, córtex orbito-frontal, temporal basal e mesial, pré-cúneo e cíngulo anterior e posterior foi determinada pelo efeito sinérgico (interação) entre a densidade de agregados de β-amiloide e o grau de desintegracão do fascículo angular, obtidos via [^{18}F]Florbetapir e FA, respectivamente. Não foram identificados clusters estatisticamente significativos nos grupos CN e CCL.

Estes resultados apóiam o conceito de que o efeito sinérgico, mais do que os efeitos independentes da amiloidose e da desintegração da substância branca, determina o hipometabolismo regional na DA. De fato, o efeito da interação em nosso modelo, envolvendo o depósito de β-amiloide e a desconexão da substância branca, contribui para um conceito integrativo da fisiopatologia da DA, em que a

ação combinada de diferentes processos patológicos potencializa a degeneracão do cérebro.

Palavras-chave: *Doença de Alzheimer. Biomarcadores. Tomografia por Emissão de Pósitrons. Imagem de Tensor de Difusão. Interação. Substância branca. Hipometabolismo. Demência.*

ABSTRACT

The pathophysiology of Alzheimer's disease (AD) involves several pathological mechanisms, including amyloid-β and neurofibrillary tangles deposition, white matter changes and neurodegeneration. In this tranversal study, we investigated the interaction between white matter (WM) integrity and amyloid-β deposition as a potential determinant of cerebral hypometabolism in the Alzheimer's disease (AD) continuum. Using the Alzheimer's Disease Neuroimaging Initiative (ADNI) database, ninety-six subjects (cognitively normal (CN), n = 27; mild cognitive impairment (MCI), n = 49; and AD, n = 20) had positron emission tomography (PET) with [^{18}F]Fluorodeoxyglucose ([^{18}F]FDG) and [^{18}F]Florbetapir, and magnetic resonance imaging (MRI) with Diffusion Tensor Imaging (DTI).

In the first part of the study, we identified areas of fractional anisotropy (FA) reduction in angular bundle and fornix in the AD group. Among these regions, we selected for subsequent analyses a voxel of interest (VOI) in the angular bundle bilaterally. Then, using a voxel-based interaction model we examined the association of FA in the angular bundle, amyloid-β deposition, and also the potential interaction of these variables with [^{18}F]FDG cerebral hypometabolism.

In the AD group, [^{18}F]FDG hypometabolism in the striatum, basal and mesial temporal, orbitofrontal, precuneus, anterior and posterior cingulate cortices was associated with the interaction between increase in [^{18}F]Florbetapir standardized uptake value ratio (SUVR) in regions of interest and reduction in angular bundle FA. No significant clusters were identified in CN and MCI subjects.

The interaction model, including amyloid-β deposition and WM disconnection, supports the concept of an integrative framework of AD pathophysiology where the combination of distinct pathological processes leads to progressive brain dysfunction in important cognitive-related areas.

Keywords: *Alzheimer's disease. Biomarkers. Positron Emission Tomography. Diffusion Tensor Imaging. Interaction. White Matter. Hypometabolism. Dementia.*

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Aβ – Beta Amiloide

ADNI – *Alzheimer's Disease Neuroimaging Initiative*

APOE – Apolipoproteína E

CCL – Comprometimento Cognitivo Leve

CDR – *Clinical Dementia Rating*

CN – Controles Normais

DA – Doença de Alzheimer

DTI – Imagem de Tensor de Difusão, do inglês *Diffusion Tensor Imaging*

EDSD - *European DTI Study in Dementia (EDSD)*

FA – Anisotropia Fracionada, do inglês *fractional anisotropy (sem unidade)*

[^{18}F]FDG – [^{18}F]Fluordeoxiglicose

MD – Difusividade Média, do inglês *mean diffusivity (unidade: m^2s^{-1})*

MEEM – Mini-exame do estado mental

PET – Tomografia por Emissão de Pósitrons, do inglês *positron emission tomography*

PUCRS – Pontifícia Universidade Católica do Rio Grande do Sul

RMN – Ressonância magnética nuclear

SUVR – valor de captação padronizado, do inglês *Standardized Uptake Value Ratio (sem unidade)*

VOI – Voxel de interesse, do inglês *voxel of interest*

SUMÁRIO

1 INTRODUÇÃO

1.1 APRESENTAÇÃO

O interesse pela Doença de Alzheimer (DA) surgiu durante a residência em neurologia no Hospital São Lucas da Pontifícia Universidade Católica do Rio Grande do Sul (PUCRS), onde tive meu primeiro contato com pacientes com demências e percebi o grande impacto que essa condição clínica traz para o paciente e seu contexto familiar e social. Sob supervisão do Prof. André Palmini, comecei a me aproximar da área de neurologia cognitiva e a perceber os seus desafios. Ao mesmo tempo em que a incidência e a prevalência da DA cresciam globalmente, via de regra o diagnóstico era realizado de forma tardia, oferecendo perspectivas modestas de resposta aos tratamentos.

Biomarcadores de neuroimagem tem permitido o estudo detalhado da fisiopatologia da progressão da doença, e através de exames como a tomografia por emissão de pósitrons (PET), diversos processos previamente descritos apenas do ponto de vista histopatológico passaram a ser analisados de forma dinâmica *in vivo*. Este contexto científico internacional refletia-se também na PUCRS, onde o Prof. Jaderson Costa da Costa projetava o Instituto do Cérebro do Rio Grande do Sul (InsCer) dentro desse conceito e buscava aplicar a neuroimagem funcional como um recurso para obter avanços na área da neurociência. Neste período, comecei a atuar também junto ao Prof. Carlos Rieder no Ambulatório de Demências e Distúrbios do Movimento Atípicos, passando a ter contato com os diferentes espectros clínicos de tais condições e vivenciando as dificuldades no diagnóstico preciso e tratamento dessas enfermidades.

Concomitantemente com a idéia clínica de especialização em neurologia cognitiva, surgiu também o indissociável interesse cientifico pelo tema. Neste período conheci o Prof. Pedro Rosa-Neto, da McGill University, cuja relação de longa data com a PUCRS o trazia a Porto Alegre para ministrar atividades de extensão a respeito da neuroimagem na DA, o que aumentou meu interesse acerca do assunto. Esse contato levou a uma crescente aproximação dos Profs. Pedro Rosa-Neto e André Palmini e também a buscar realizar doutorado no tema. Através do prêmio “Emerging Leaders in the Americas” do *Canadian Bureau of Foreign Students*, surgiu a oportunidade de realizar parte do meu doutorado e especialização

em neurologia cognitiva junto ao Dr. Pedro Rosa-Neto e Dr. Serge Gauthier no *McGill Center of Studies in Aging*. Durante o período em que estive na McGill University em Montreal, dividi inicialmente meu tempo entre atividades assistenciais no Douglas Hospital, sob supervisão do Dr. Pedro Rosa-Neto e do Dr. Serge Gauthier e atividades científicas focadas em neuroimagem na DA. No *Translational Neuoimaging Laboratory* da McGill University, tive contato com o consórcio internacional *ADNI (Alzheimer's Disease Neuroimaging Initiative)*, projeto colaborativo entre diversas universidades da América do Norte, onde pacientes idosos são seguidos longitudinalmente dentro do *continuum* da DA através de avaliações clínicas e de neuroimagem. O formato do ADNI é particularmente interessante, pois os dados ali gerados têm permitido a realização de relevantes pesquisas com exames de neuroimagem – de grande custo – e dentro de um formato padronizado que impactaram significativamente a pesquisa científica no tema.

O período em Montreal trouxe um amadurecimento clínico-científico, oportunizando grande aprendizado na área. Este processo levou a contribuir de forma original no tema através da pesquisa aqui apresentada. Neste estudo, procurei integrar diferentes modalidades de neuroimagem para investigar o efeito da interação entre distintos processos patológicos na DA. Através de um modelo estatístico de neuroimagem, testei a hipótese de que a interação entre os processos de deposição de amiloide e o comprometimento da substância branca está associada com o hipometabolismo cerebral na DA .

Esta tese traz o resultado dessa trajetória de aprendizado. Os artigos que encontram-se na sessão de anexos constituem a base desta dissertação e refletem o processo de construção e amadurecimento científico no tema. Como as diversas facetas da integração de biomarcadores de imagem na DA foram detidamente revisadas nos artigos em anexo, a revisão teórica desta dissertação apenas apresenta os aspectos fundamentais para a compreensão do estudo original. Por outro lado, a metodologia e o resultados descritos baseiam-se no estudo original também em anexo e submetido para publicação.

1.2 ASPECTOS GERAIS

Nos últimos dois séculos, foram observadas importantes mudanças sociais com um exponencial crescimento da expectativa de vida da população (1). Tal fenômeno tem gerado importantes alterações nas pirâmides populacionais em todo o mundo, com um progressivo aumento da população idosa. Dados do Global AgeWatch de 2015 apontam que há mais de 900 milhões de indivíduos no mundo com mais de 60 anos, e até 2030 esse número deve chegar a 1.4 bilhão de pessoas (https://www.ageinternational.org.uk).

Uma das consequências mais relevantes desse processo demográfico é o aumento na incidência e prevalência das doenças ligadas ao envelhecimento, período em que ocorre um acúmulo de modificações deletérias em células e tecidos que levam ao maior risco de desenvolvimento de doenças e morte (2). Nesse cenário, observa-se um aumento na incidência das condições biológicas chamadas de neurodegenerativas, caracterizadas por perda progressiva de neurônios no sistema nervoso. Dentre as condições neurodegenerativas, existem alguns quadros que podem levar ao desenvolvimento de demência, estágio clínico caracterizado por comprometimento da funcionalidade e autonomia do indíviduo (3).

A doença de Alzheimer (DA) é a principal condição associada com o comprometimento cognitivo, e apresenta grande impacto para o indivíduo acometido por essa enfermidade e para a família e a sociedade onde está inserido. Frente a esta preocupante conjuntura, observa-se um crescente interesse da comunidade científica na busca por uma melhor compreensão de sua fisiopatologia, visando ao desenvolvimento de terapias mais efetivas e modificadoras da história natural da doença.

1.3 HISTÓRIA

O termo demência deriva da palavra latina *demens*, que é composta do prefixo *de* (sem ou não) e do termo *mens* (mente). Essa palavra passou a ter conotação médica no início do século XVIII através de Jean Etienne Esquirol (4), porém a condição clínica de deterioro cognitivo associado ao envelhecimento é bastante conhecida desde a antiguidade. Desde o Egito e Grécia Antiga, já existem registros descrevendo e formulando os primeiros conceitos de demência, mas foi no período greco-romano que Galeno caracterizou a demência como um distúrbio mental (5).

Apesar das diversas referências a respeito da idéia de demência ao longo da história, o conhecimento específico sobre DA é relativamente recente. Em 1906, em um encontro alemão de psiquiatria na cidade alemã de Tübingen, o psiquiatra alemão Alois Alzheimer (1864-1915) descreveu pela primeira vez as características clínicas e patológicas de uma então chamada demência pré-senil (6). Na sua descrição, Alzheimer descreveu o caso da paciente Auguste Deter, cujo quadro clínico foi acompanhado por ele entre 1901 e 1906. A paciente havia sido internada em um asilo municipal em Frankfurt, levada pelo marido devido a alterações neuropsiquiátricas manifestadas a partir dos 51 anos. Segundo o marido, Auguste vinha apresentando importantes alterações na sua personalidade, com delírios paranóides associados a um severo comprometimento da memória e prejuízo funcional na realização de suas atividades de vida diária. Durante o período em que esteve hospitalizada, Auguste apresentava também desorientação temporo-espacial, e com o passar dos anos seu estado geral deteriorou progressivamente: a fala tornou-se inteligível e, em seu último ano de vida, permaneceu acamada. Auguste Deter faleceu em 1906, pouco antes de completar 56 anos, por septicemia decorrente de úlceras de pressão resultantes do longo período em decúbito dorsal.

Na data da morte de Auguste Deter, Alois Alzheimer estava trabalhando em Munique, permanecendo ainda extremamente interessado pelo caso em questão. Após o óbito de Auguste, Alois Alzheimer solicitou que lhes fossem enviados os registros clínicos e o cérebro da paciente para sua análise. O exame realizado *post-mortem* revelou a deposição de "substâncias peculiares" no córtex cerebral, atualmente caracterizados como a presença de β-amiloide, bem como uma massiva perda neuronal. Os emaranhados neurofibrilares foram descritos posteriormente por Fuller, pupilo e colaborador de Alois Alzheimer. Em 1906, Alzheimer apresentou seus dados no encontro alemão de psiquiatras em Tübingen, e logo após relatou tais achados em um artigo chamado *''A characteristic serious disease of the cerebral cortex'',* em 1907 (7)*.* Em 1911, Dr. Alzheimer abordou de forma mais abrangente suas observações em outro artigo, no qual incluiu também os dados de outro paciente com características clínicas muito semelhantes a Auguste Deter. Neste artigo, Alois Alzheimer incluiu também ilustrações demonstrando a presença de placas de amiloide e de emaranhados neurofibrilares **(Figura 1).**

O termo Doença de Alzheimer (DA) foi introduzido pela primeira vez em 1910, pelo proeminente psiquiatra Emil Kraepelin, na oitava edição de seu Manual de

Psiquiatria, no capítulo de demências senis e pré-senis. Neste livro, o autor abordou as características clínicas e histológicas de “um grupo peculiar de casos com alterações celulares severas” descrita por Alois Alzheimer poucos anos antes.

Figura 1. Ilustração de Alois Alzheimer de 1911, demonstrando a presença de emaranhados neurofibrilares e de placas de amiloide.

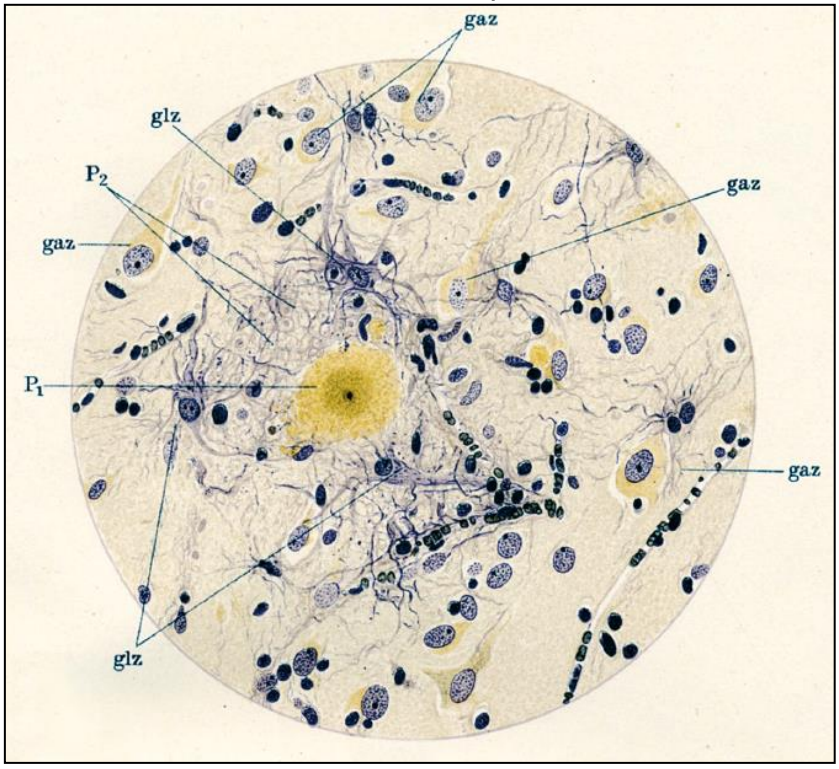


Figura adaptada de Dahm e col. Current biology (2006). (7).

1.4 EPIDEMIOLOGIA

A DA é atualmente reconhecida como a principal causa de demência no mundo, estimando-se uma prevalência de cerca de 35 milhões de pessoas globalmente. Nos Estados Unidos da América (EUA), a doença acomete um em cada nove indivíduos com mais de 65 anos de idade, e acima de 85 anos a prevalência é superior a 37% (8). Com o progressivo aumento da expectativa de vida, projeções estimam que ocorra uma duplicação desses índices a cada 20 anos nas próximas décadas, atingindo números ainda mais alarmantes.

O maior estudo populacional brasileiro foi realizado na cidade paulista de Catanduva, onde observou-se uma prevalência de demência de 7.1% na população acima de 65 anos, sendo que 55.1% dos casos correspondiam a DA e 14.4% a patologia mista (DA + doença cerebrovascular) (9). Na avaliação subsequente, em

que foram reavaliados os indivíduos sem diagnóstico de demência, observou-se que a taxa de incidência de demência praticamente dobra a cada 5 anos, com uma maior incidência em mulheres e uma tendência a ocorrer mais em analfabetos (10). Através dos dados atuais demográficos brasileiros, estima-se uma prevalência de DA de 1,2 milhão de pessoas no país, com uma incidência média de 100 mil novos casos por ano.

1.5 CONCEITOS E MODELOS ATUAIS – A DA COMO UM *CONTINUUM*

Segundo o critério diagnóstico de 1984, o termo DA contemplava apenas pacientes em estágios com claro comprometimento funcional, chamado de demência, e o diagnóstico definitivo exigia achados histopatológicos compatíveis com DA (11). O conceito de DA foi revisado em 2011, quando, após avanços na compreensão da doença, a DA passou a ser conceitualizada como um *continuum* que envolve diversos processos neurobiológicos progressivos e que se apresenta através de diferentes estágios clínicos (12). Esse *continuum* estende-se desde o funcionamento cognitivo absolutamente normal no idoso até alterações leves e progressivamente mais graves de memória, de funções executivas e outras esferas da cognição, atingindo um estágio de significativo comprometimento cognitivo e funcional (13).

Atualmente, a fisiopatologia da DA tem sido descrita através de uma complexa sequência de eventos neuropatológicos, iniciando com a deposição de β-amiloide e seguida pela hiperfosforilação e acúmulo da proteína tau (14, 15). Nos estágios mais avançados do modelo atual da doença, quando observa-se neurodegeneração, ocorre declínio metabólico, estrutural e clínico (13).

Sob o ponto de vista clínico, os recentes avanços na compreensão da DA e a idéia de tratar-se de um processo dinâmico, desenvolvido durante décadas, levaram a importantes mudanças conceituais na avaliação e classificação clínica dos pacientes. Um dos exemplos que ilustra estes avanços conceituais diz respeito a indivíduos que, à avaliação, apresentam comprometimento em algum dos domínios cognitivos sem impacto significativo na sua funcionalidade. Estes passaram a ser classificados através da terminologia Comprometimento Cognitivo Leve (*em inglês mild cognitive impairment – MCI*) (16).

O entendimento de que a demência representa os estágios finais deste *continuum* foi um avanço fundamental, uma vez que permite uma projeção a respeito deste desfecho indesejável enquanto o paciente ainda está funcionando relativamente bem, com graus variáveis de independência e portanto nas fases mais iniciais do espectro. Através da elaboração desses novos conceitos e do desenvolvimento e incorporação do uso de biomarcadores que refletem os processos patológicos da DA, os critérios diagnósticos foram revisados e passaram a incluir as diferentes fases clínicas e de categorias diagnósticas da doença (3, 17, 18) **(Figura 2)** .

As importantes mudanças dos paradigmas na área geraram novas questões e desafios, como a busca pelo desenvolvimento de intervenções terapêuticas realmente eficazes, a fim de evitar ou limitar a progressão da doença. Para que isso seja possível, é altamente desejável que possamos encontrar meios de identificar aqueles indivíduos idosos que irão beneficiar-se destas intervenções, ou seja, aqueles nos quais o tratamento iniciará de forma mais preventiva do que paliativa, sendo administrado antes que ocorra perda funcional significativa. Nesta direção, as pesquisas no tema têm buscado alterações sutis e precoces na estrutura e na dinâmica funcional do cérebro de idosos, passando a incluir em estudos como o ADNI pacientes cognitivamente normais e com comprometimento cognitivo leve.

Figura 2. Modelo atual hipotético dinâmico do *continuum* da DA através de biomarcadores.

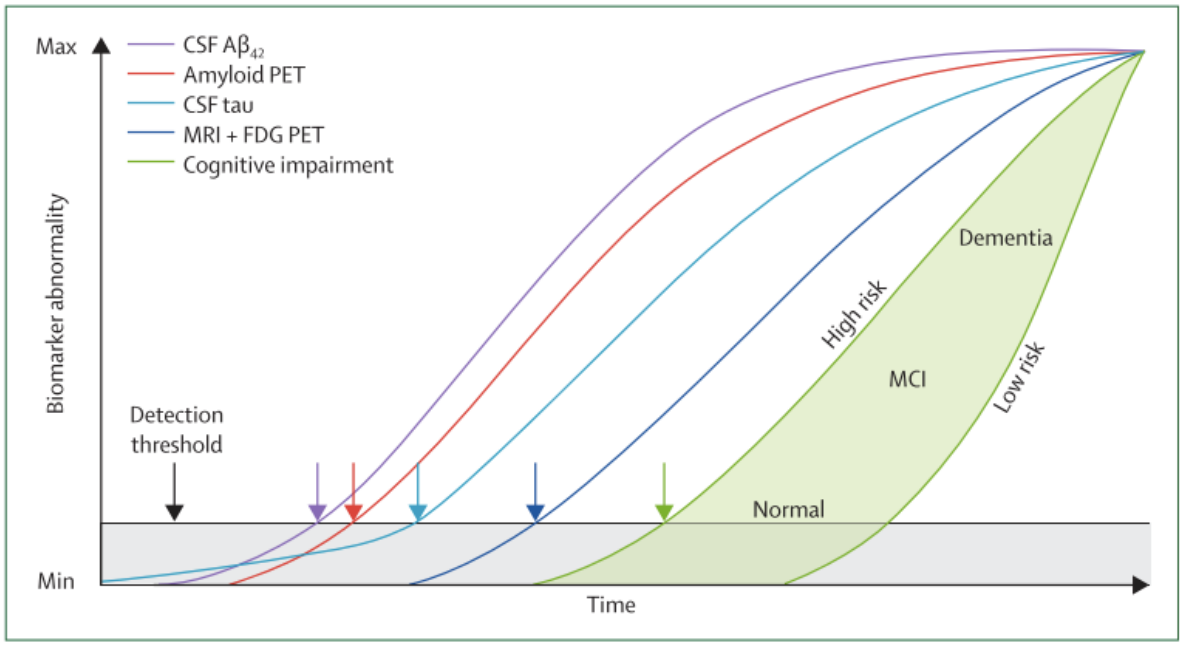


Figura adaptada de Jack e col. Lancet neurology (2013).(15)

1.6 FISIOPATOLOGIA

A fisiopatologia da DA envolve diversos mecanismos patológicos, sendo os principais o acúmulo de β-amiloide e de emaranhados neurofibrilares compostos pela proteína tau hiperfosforilada. Tais alterações estão associadas a diversos eventos, incluindo neurodegeneração, neuroinflamação com alterações na substância branca e disfunção de diferentes neurotransmissores **(Figura 3).**

Figura 3. Ilustração de eventos fisiopatológicos na DA.

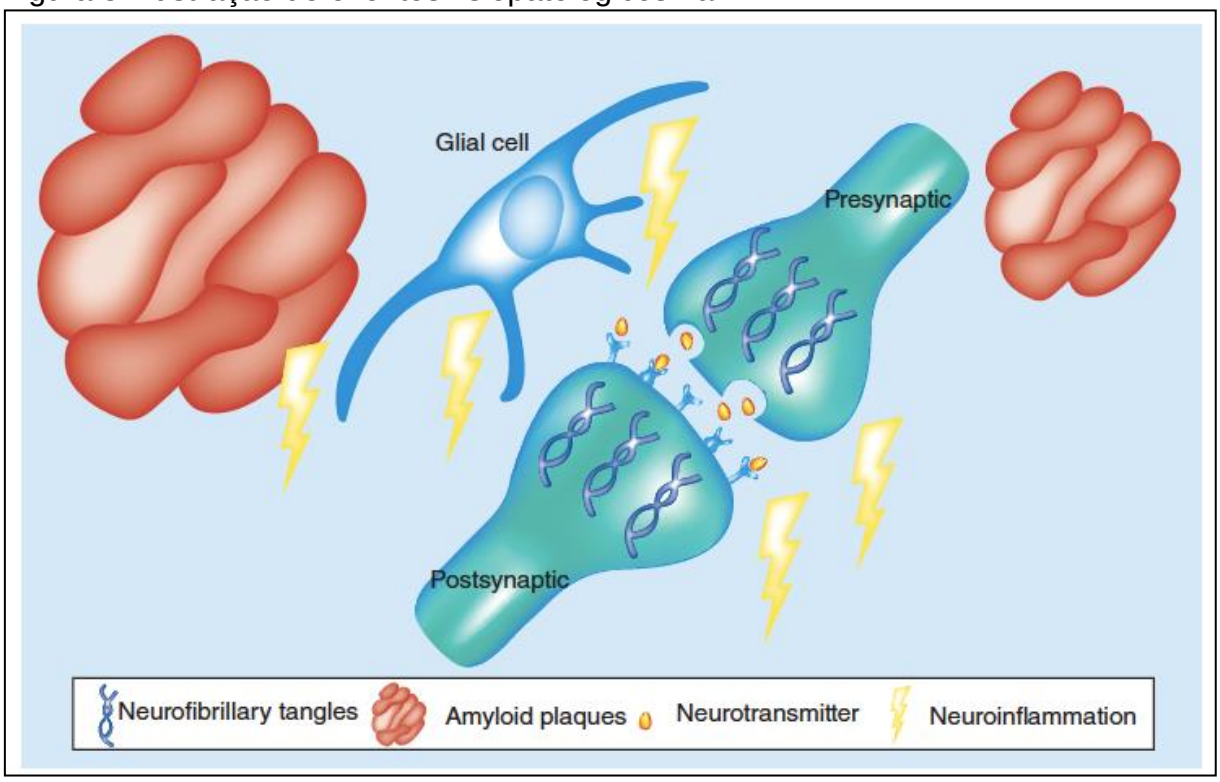


Figura adaptada de Schilling e col. Future Neurology (2014). (19).

1.6.1 β-amiloide

A deposição extracelular de placas de β-amiloide é uma das principais características da DA, presente inclusive na descrição inicial de Alois Alzheimer. O acúmulo de β-amiloide em placas é a última etapa do processo amiloidogênico iniciado pela quebra da proteína precursora do amiloide (APP) através de duas

enzimas: β-secretase e γ -secretase, levando à formação de peptídeos de β -amiloide, sendo os mais comuns o Aβ1-40 e o Aβ1-42 (20). Os peptídeos passam por um processo de oligomerização e fibrilogênese, formando então placas maduras e insolúveis de β-amiloide. Conceitos atuais apontam que as formas mais tóxicas de β-amiloide são os oligômeros e indicam que estes estão associados com a disfunção sináptica da doença (21). Outra característica importante é que os depósitos de β-amiloide induzem à hiperfosforilação da proteína tau, processo a ser discutido na próxima seção.

Os depósitos de β-amiloide ocorrem de forma progressiva e, segundo a descrição de Braak, podem ser separados em três estágios: A) há o envolvimento das porções basais do isocórtex; B) as alteração ocorrem nas áreas associativas do isocórtex; e C) há comprometimento de praticamente todas as áreas do isocórtex (22). No estágio C, outras áreas além do córtex estão também envolvidas, como o corpo estriado, tálamo e hipotálamo.

1.6.2 A proteína tau

A proteína tau tem como principal função a estabilização dos microtúbulos, que constituem o citoesqueleto dos axônios das células nervosas (23). A regulação da fosforilação da tau ocorre através de enzimas chamadas proteínas cinases, como glicogênio sintase cinase 3-beta (GSK-3β, do inglês “glycogen synthase kinase 3β”), ciclina dependente de cinase 5 (CDK5, do ingles “cyclin-dependent kinase 5”), proteína cinase dependente de cAMP (PKA, do inglês “cAMP-dependent protein kinase”) e proteína reguladora associada a microtúbulo (MARK, do “microtubule-affinity-regulating-kinase”) (24). A sinalização e plasticidade neuronal também estão associadas a fosforilação da proteína tau, e alterações nestes processo são parte fundamental da fisiopatologia da DA (25, 26).

Um dos mais importantes processos fisiopatógicos da DA é a fosforilação exacerbada da proteína tau, desencadeando instabilidade dos microtúbulos e neurodegeneração. Através dessa hiperfosforilação ocorre a formação e depósitos intracelulares chamados de emaranhados neurofibrilares (27-29). A propagação cerebral da deposição dos emaranhados neurofibrilares ocorre também dentro de seis estágios padronizados por Braak, sendo: I-II) camada transentorrinal; III – IV) regiões do sistema límbico e V – VI) áreas isocorticais (22).

Apesar do importante papel da agregação da proteína tau na DA, tais alterações não são observadas apenas na DA, mas também em outras doenças neurodegenerativas. Tais condições, denominadas taupatias, são responsáveis por doenças que apresentam-se com diferentes fenótipos clínicos, como demência frontotemporal e paralisia supranuclear progressiva (23).

1.6.3 Outros mecanismos

Desde a sua descrição original, a DA é caracterizada como uma doença da substância cinzenta (6), e as mais proeminentes teorias sobre a fisiopatologia da doença postulam que o acúmulo das proteínas amiloide e tau justificam a maioria dos processos neurobiológicos observados no quadro (22, 30). Entretanto, a presença de respostas inflamatórias foi também descrita, tanto através do aumento de citocinas pró-inflamatórias e de fator de necrose tumoral, como pela ativação glial e presença de fatores de complemento junto às placas de amiloide e dos emaranhados neurofibrilares (31-37). A neuroinflamação, apesar de inicialmente ocorrer como mecanismo reativo e protetor no sistema nervoso central, contribui para a disfunção sináptica e neurodegeneração (38-40).

A degeneração da substância branca na DA foi observada em análises histopatológicas desde estágios pré-clínicos (41). Através de estudos de neuroimagem, cujos métodos serão discutidos na sequência, foi descrito o comprometimento de diversos feixes desta substância, sugerindo que tais alterações também fazem parte da fisiopatologia da doença (42-46). Apesar do conceito atual do envolvimento da substância branca como parte do processo fisiopatológico da DA, o mecanismo subjacente a esse comprometimento ainda não está claramente definido, e diferentes teorias buscam explicar esse processo, sendo as principais a Degeneração Walleriana e a Retrogênese. Na teoria da Degeneração Walleriana, o conceito é que o comprometimento da substância branca ocorre de forma secundária à degeneração do córtex. Já na teoria da Retrogênese, a idéia é que esse processo ocorre de forma primária nessas estruturas (47, 48). No modelo da Retrogênese, o dano primário na mielina e o comprometimento axonal levam à atrofia da substância branca, sendo mais sensíveis as regiões com axônios cortico-corticais de menor diâmetro, especialmente nas áreas neocorticais e lobo temporal (49).

Dentro do processo patológico da DA, ocorre a disfunção de diversos neurotransmissores. O glutamato é o principal neurotransmissor excitatório cerebral, e os oligômeros de β-amiloide interagem de forma deletéria com os receptores glutamatérgicos, levando a um processo de excitoxicidade (50). Nesse processo, através da indução da abertura do canal inotrópico receptor N-metil-D-Aspartato (NMDA), há um aumento no influxo de cálcio, ativação de vias de sinalização associadas com a fosforilação da proteína tau e morte neuronal (51). Este mecanismo justifica o uso da Memantina em fases moderadas a avançadas da doença, uma vez que essa medicação realiza um bloqueio de baixa afinidade nos receptores NMDA, reduzindo o influxo de cálcio e mantendo a atividade do receptor de forma adequada (52-54).

O sistema colinérgico também sofre comprometimento na DA, ocorrendo redução nos níveis de acetilcolina associada à redução na atividade da enzima colina-acetil-transferase, responsável pela síntese de acetilcolina (55, 56). Tais alterações justificam o uso clínico de medicações inibidoras das colinesterases a fim de reduzir a degradação da acetilcolina e obter melhora em funções cognitivas. Alterações no sistema serotoninérgico também foram descritas na DA, estando associadas a alterações cognitivas e neuropsiquiátricas (57-59).

1.7 A NEUROIMAGEM NA INVESTIGAÇÃO *IN VIVO* DA PATOLOGIA DA DOENÇA DE ALZHEIMER: O ADVENTO DOS BIOMARCADORES

O advento de marcadores mensuráveis *in vivo* para a DA, os chamados biomarcadores, tem permitido importantes avanços na compreensão dos eventos patológicos observados na doença, assim como tem sido útil no diagnóstico da doença e monitoramento de resposta a intervenções clínicas. Os biomarcadores hoje disponíveis podem ser obtidos através de neuroimagem ou do líquido cefalorraquidiano com análise dos níveis de amiloide β_{1-42} e da proteína tau total e fosforilada. Os marcadores obtidos no líquido cefalorraquidiano apresentam boa acurácia diagnóstica, entretanto tem como fator limitante a necessidade da realização de punção lombar para coleta (60).

O desenvolvimento dos métodos de neuroimagem durante as últimas décadas trouxe importantes avanços clínico-científicos na área. Os exames de neuroimagem podem ser classificados como estruturais, quando avaliam essencialmente a

morfologia cerebral, e exames funcionais, nos quais processos metabólicos são quantificados de forma dinâmica (61). Os exames estruturais são importantes na investigação de quadros demenciais, uma vez que a Ressonância Magnética Nuclear (RMN) permite avaliar eventuais assimetrias inter-hemisféricas, atrofia lobar e lesões vasculares associadas ao quadro, especialmente através da sequência FLAIR *(fluid attenuated inversion recovery)* (62). Na DA, a avaliação de atrofia cortical e especialmente dos hipocampos também contribuem no processo de investigação diagnóstica (63).

A Tomografia por Emissão de Pósitrons (PET), um dos principais exames funcionais de neuroimagem, será abordado na sequência, bem como outras modalidades de RMN.

1.7.1 Tomografia por Emissão de Pósitrons

Através de exames de neuroimagem, como a tomografia por emissão de pósitrons (PET), pode-se obter diferentes informações de distintos processos cerebrais *in vivo* **(Figura 4).** No exame de PET, através de radiotraçadores como [^{18}F]Fluordeoxiglicose ([^{18}F]FDG) e [^{11}C]Pittsburgh Compound B ([^{11}C] PIB) ou [^{18}F]Florbetapir, é possível quantificar metabolismo e depósito de amiloide cerebral **(Figura 5),** respectivamente (19, 64). A quantificação do exame de PET cerebral é realizada através de um índice chamado SUVR (*Standardized Uptake Value Ratio*), calculado através de uma razão entre o valor de captação do traçador em um ponto especifico e um ponto de referência, como cerebelo ou ponte. Na DA, através do PET, observa-se de forma notória hipometabolismo cerebral e deposição de amiloide em áreas como o córtex do cíngulo posterior e áreas temporo-parietais associativas (65, 66) **(Figura 6)** .

Iniciativas como o consórcio internacional ADNI permitiram grandes avanços científicos através de informação geradas pelos exames de PET, e hoje critérios internacionais para pesquisa em DA já incluem a utilização desta ferramenta (18).

Figura 4. Processos envolvidos na realização do exame de PET, desde a síntese no cíclotron até a aquisição da imagem.

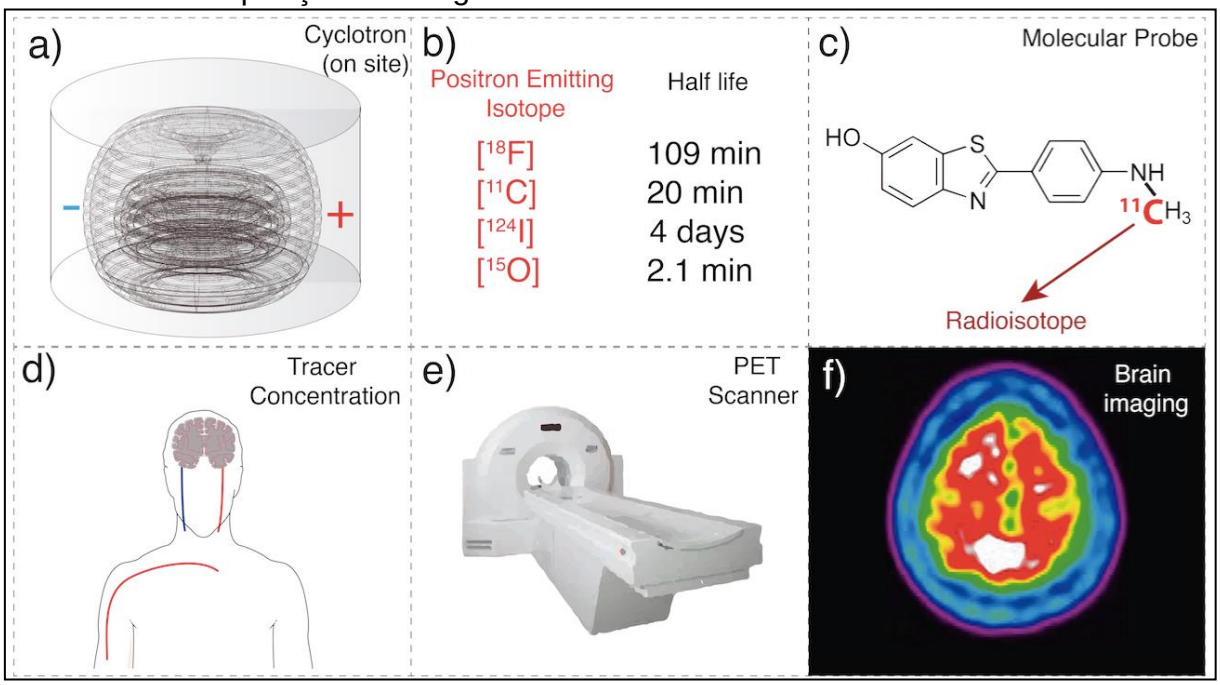
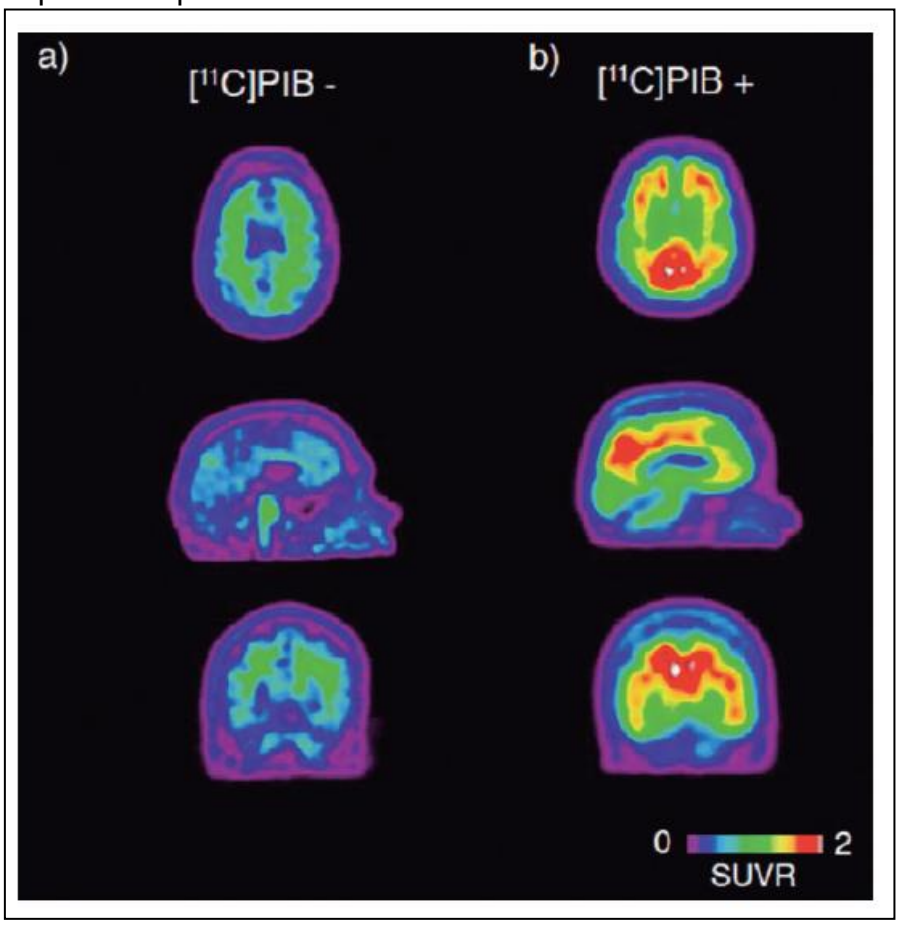


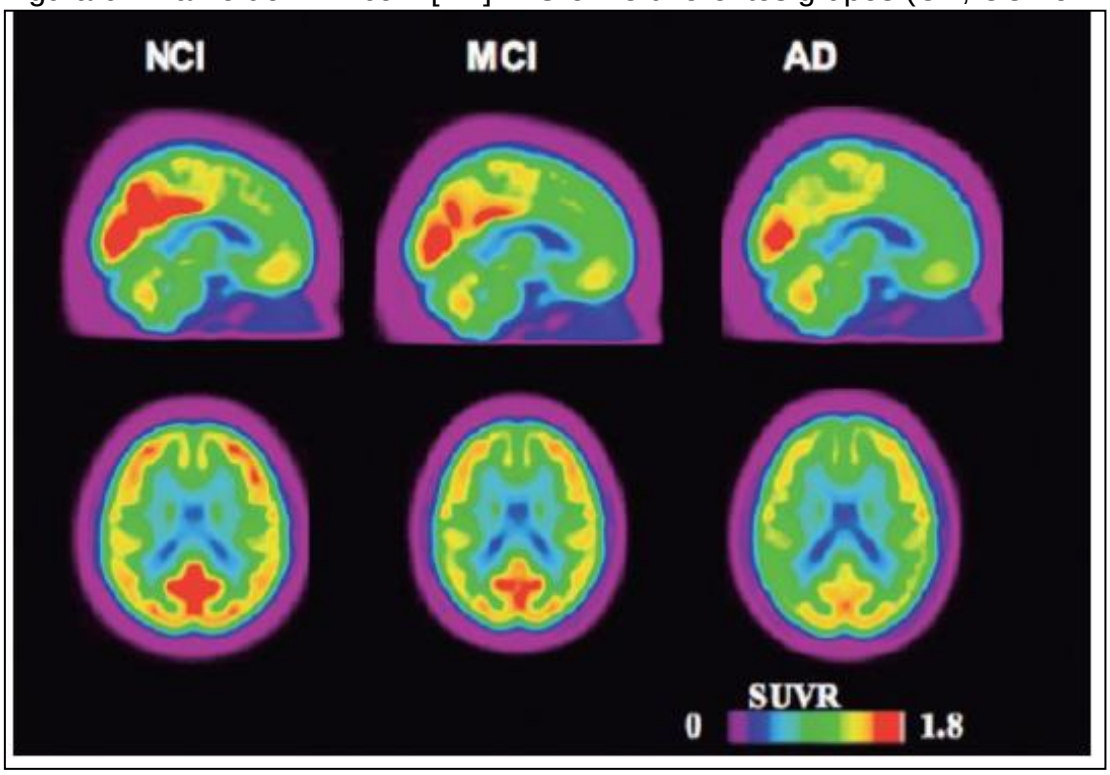
Figura adaptada de Schilling e col. Future Neurology (2014).(19)

Figura 5. Exame de PET com [^{11}C]Pittsburg Compound B, radiotraçador para depósito de β-amiloide.



a) paciente com síndrome cortico-basal sem evidência de depósito de β-amiloide; b) paciente com DA com evidência de extensa deposição de β-amiloide, de forma marcada no cíngulo posterior, pré-cúneo, córtex pré-frontal e parietal inferior.
Figura adaptada de Schilling e col. Dementia & Neuropsychologia (2016).(64)

Figura 6. Exame de PET com [^{18}F]FDG em 3 diferentes grupos (CN, CCL e DA).



Indivíduos cognitivamente normais (NCI), pacientes com Comprometimento Cognitivo Leve (CCL) e pacientes com DA. No grupo DA, observa-se importante hipometabolismo no cíngulo posterior e pré-cunêo, assim como no córtex frontal e parietal inferior.
Figura adaptada de Schilling e col. Dementia & Neuropsychologia (2016).(64)

1.7.2 RMN com Imagem por Tensor de Difusão

A imagem por tensor de difusão (DTI) é uma sequência de Ressonância Magnética Nuclear (RMN) que fornece informações sobre a microestrutura da substância branca através de diversos índices, como Anisotropia Fracionada (FA) e Difusividade Média (MD) **(Figura 7)**. Este método permite determinar a organização tecidual das fibras da substância branca, através da análise da difusividade da água no tecido cerebral de forma quantitativa. A FA, o índice mais específico gerado pelo DTI para análise da substância branca, é expresso através de valores entre zero e um, refletindo o ordenamento das moléculas baseado no quão direcional é a difusão da água, sendo 0 para difusão isotrópica e 1 para totalmente direcional (67). Uma redução neste índice reflete a perda de mielina nos axônios, e, portanto, perda do efeito direcional da difusão da água (menor anisotropia), expressando comprometimento da substância e da conectividade estrutural (68, 69) **(Figura 8)**.

Diversos estudos demonstraram danos em feixes da substância branca através da técnica DTI, especialmente em estruturas relacionadas ao sistema límbico e de

memória (70, 71). Em pacientes com DA, foram observadas redução na FA e aumento da MD nas principais áreas envolvidas na DA, incluindo corpo caloso, lobo temporal medial e lateral, fórnix, giro do cíngulo, pré-cúneo e substância branca do lobo pré-frontal.

Figura 7. Representação das medidas quantitativas em um feixe de substância branca obtidas através da imagem por tensor de difusão.

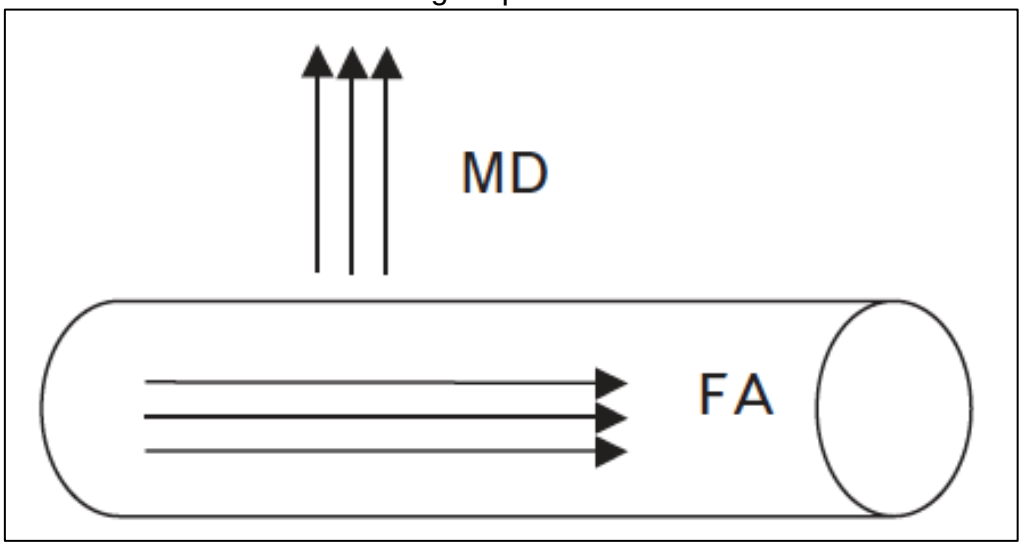
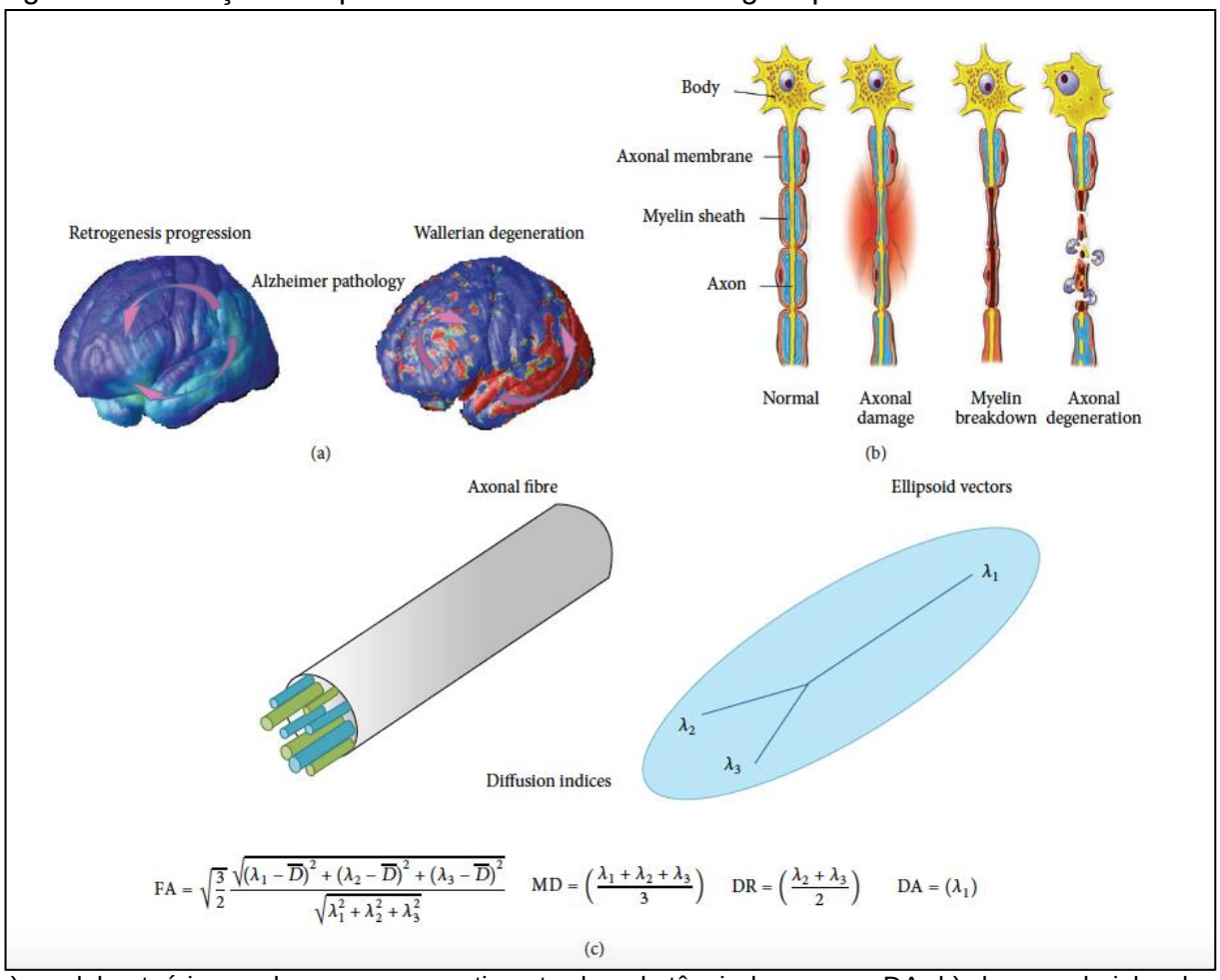


Figura adaptada de Chua e col. Current opinion in neurology (2008).(69)

Figura 8. Ilustração dos processos associados a imagem por tensor de difusão.



a) modelos teóricos sobre o comprometimento da substância branca na DA; b) dano na bainha de mielina associado ao processo patológico da doença; c) índices calculados através da imagem por tensor de difusão.
Figura adaptada de Alves e col. BioMed research international (2015).(49)

1.8 INTERAÇÃO ENTRE PROCESSOS FISIOPATOGÊNICOS NA DOENÇA DE ALZHEIMER

Estudos recentes têm integrado diferentes biomarcadores de neuroimagem para melhor compreender as relações entre os diferentes processos fisiopatológicos da DA. Tem-se tentado compreender, sobretudo, quais mecanismos contribuem para o hipometabolismo cortical na DA. A deaferentação por lesão em feixes de substância branca e a deposição de β-amiloide são dois dos mecanismos estudados e prioritariamente relacionados a esta redução metabólica observada na progressão do quadro.

Em um estudo experimental com primatas, Meguro e col. demonstraram que lesões nos córtices peririnal e entorrinal são seguidas por hipometabolismo

hipocampal e no neocórtex (72). Estudos subsequentes relacionaram os feixes de substância branca comprometidos com o metabolismo de regiões cerebrais interconectadas (por exemplo: desconexão no fórnix e hipometabolismo do cíngulo posterior) (73). Outros estudos relacionam a progressão regional do hipometabolismo cerebral com o depósito de amiloide nestas áreas (74).

Através de modelos criados especificamente para a análise integrada de diferentes biomarcadores, Pascoal e col. sugerem que a interação entre o acúmulo de β-amiloide e da proteína tau apresenta efeito determinante na redução metabólica na DA (75). Tais achados tem fundamental importância na fisiopatologia da DA, uma vez que o modelo atual da doença envolve um cascata seqüencial de eventos. O conceito integrativo e sinérgico entre diferentes mecanismos vem sendo amplamente debatido, entretanto os efeitos da interação entre o acúmulo de β-amiloide e da proteína tau foram observados apenas em indivíduos cognitivamente normais e com CCL. Assim, esses resultados sugerem o possível envolvimento de outros mecanismos nas alterações observadas especificamente na fase de demência na DA. Dessa forma, no presente estudo buscamos avaliar, através de um modelo de interação, o efeito da desconexão do fascículo angular, da deposição de amiloide e da interação entre ambos no hipometabolismo cerebral na DA.

2 JUSTIFICATIVA

A incidência de DA vem apresentando um progressivo aumento nas últimas décadas, e não possui ainda um tratamento curativo ou modificador de sua história natural. Um dos principais fatores limitantes para o desenvolvimento de novas terapias é o diagnóstico ainda tardio, bem como a dificuldade na identificação e compreensão de todos os mecanismos patológicos envolvidos na sua progressão.

Buscamos aqui avaliar o papel de diferentes biomarcadores na investigação do *continuum* da DA, especificamente indicadores da transição entre cognição normal e CCL e entre CCL e DA estabelecida. Quando os biomarcadores são analisados de forma isolada, podem não apresentar diferenças entre grupos com perfis cognitivos distintos ao longo do *continuum*, mas a interação entre eles pode trazer uma nova perspectiva nesta distinção. Através de exames de neuroimagem, buscamos identificar o efeito da interação entre diferentes mecanismos fisiopatológicos, como o comprometimento da substância branca e o depósito de amiloide no metabolismo cerebral destes indivíduos.

Assim, nos propusemos a investigar os efeitos de diferentes processos fisiopatogênicos na DA visando a contribuir no desenvolvimento de novas estratégias de investigação da progressão de disfunção cognitiva no envelhecimento. Especificamente, buscamos utilizar estas técnicas afim de identificar se o hipometabolismo presente na DA é secundário à desconexão do córtex, à presença de amiloide no córtex ou à interação entre ambos.

3 OBJETIVOS

3.1 OBJETIVO GERAL

Avaliar biomarcadores de neuroimagem relacionados a distintos mecanismos fisiopatogênicos, tanto de forma isolada como interativa, em idosos distribuídos em 3 pontos ao longo do *continuum* cognitivo: aqueles cognitivamente normais, indivíduos com CCL e pacientes com DA.

3.2 OBJETIVOS ESPECÍFICOS

- Avaliar:
  - diferenças na FA em distintos feixes de substância branca, comparando indivíduos com CN x DA, CN x CCL e CCL x DA;
  - a FA no voxel de interesse selecionado (fascículo angular bilateral) em todos os participantes, para obter os valores médios em cada grupo (CN, CCL, DA);
  - o SUVR global do PET [^{18}F]Florbetapir em todos os participantes, para obter os valores médios em cada grupo (CN, CCL, DA);
  - o SUVR global do PET [^{18}F]FDG em todos os participantes, para obter os valores médios em cada grupo (CN, CCL, DA);
  - diferenças entre os grupos (CN x DA, CN x CCL, CCL x DA) na FA do voxel de interesse selecionado bilateralmente no fascículo angular;
  - diferenças entre os grupos (CN x DA, CN x CCL, CCL x DA) no SUVR global de PET [^{18}F]Florbetapir;
  - diferenças entre os grupos (CN x DA, CN x CCL, CCL x DA) no SUVR global de PET [^{18}F]FDG.
- Investigar a associação entre FA no fascículo angular bilateral, o depósito de β-amiloide através do PET [^{18}F]Florbetapir SUVR nas regiões de interesse e a possível associação da interação entre ambos no hipometabolismo cerebral nos 3 grupos.

4 MATERIAL E MÉTODOS

4.1 DELINEAMENTO

Estudo transversal a partir da base de dados ***Alzheimer's Disease Neuroimaging Initiative (ADNI)***.

4.2 DEFINIÇÕES CLÍNICAS DO ESTUDO

De acordo com os protocolos do ADNI, o diagnóstico de DA foi baseado nos critérios do National Institute of Neurological and Communicative Disorders and Stroke/Alzheimer's Disease and Related Disorders Association (NINCDS/ADRDA), e a severidade do comprometimento cognitivo (DA leve, moderada ou grave) com base no escores do Mini-Exame do Estado Mental (MEEM) (76) e da escala Clinical Dementia Rating (CDR) (77). Em linhas gerais, os pacientes com DA apresentam um declínio cognitivo em relação a níveis prévios, comprometendo pelo menos dois domínios cognitivos. Esses pacientes apresentam comprometimento funcional em suas atividades, o que leva a escores de 1.0 ou mais na escala de CDR.

O ADNI2 e ADNI-GO definem como apresentando comprometimento cognitivo leve (CCL) indivíduos com queixas subjetivas de memória, cuja gravidade é objetivada através dos escores na recordação tardia de um parágrafo da escala Wechsler Memória Lógica II. Indivíduos com CCL podem ter pontuação entre 24-30 no MEEM, CDR 0.5 e preservação da funcionalidade nas atividades da vida diária (78).

4.3 DESCRIÇÃO DO BANCO DE DADOS DO CONSÓRCIO ADNI E AMOSTRA DESTE ESTUDO

Os dados utilizados na elaboração da tese e do artigo original foram obtidos a partir da base de dados do consórcio ***Alzheimer's Disease Neuroimaging Initiative (ADNI) (adni.loni.usc.edu)***. O ADNI foi lançado em 2003 por instituições norte-americanas - National Institute on Aging (NIA), National Institute of Biomedical Imaging and Bioengineering (NIBIB), Food and Drug Administration (FDA) - e

companhias privadas e organizações sem fins lucrativos, em uma parceria público-privada de U$ 60 milhões com duração de 5 anos.

O principal objetivo do ADNI foi avaliar se exames seriados de neuroimagem (RMN e PET), de outros biomarcadores e avaliações clínicas e neuropsicológicas podem ser combinados para avaliar a progressão do comprometimento cognitivo leve e DA inicial. A determinação de marcadores sensíveis e específicos de progressão na DA muito inicial destina-se a ajudar os investigadores e clínicos a desenvolver novos tratamentos e monitorar a sua eficácia, bem como diminuir a duração e os custos de ensaios clínicos.

O investigador principal desta iniciativa é o Dr. Michael W. Weiner, da Universidade da Califórnia - San Francisco, EUA. O ADNI é resultado de esforços de muitos pesquisadores e colaboradores de uma vasta gama de instituições acadêmicas e corporações privadas, e os indivíduos do estudo foram recrutados em mais de 50 centros nos EUA e Canadá. O objetivo inicial do ADNI foi recrutar 800 sujeitos, mas foi seguido pelo ADNI-GO e ADNI-2. Até o momento, estes três protocolos recrutaram mais de 1500 adultos com idades entre 55 e 90 anos, constituídos de indivíduos idosos cognitivamente normais, pessoas com transtorno cognitivo leve e pacientes com DA inicial. A duração do *follow-up* de cada grupo é especificada nos protocolos do ADNI-1, ADNI-2 e ADNI-GO. Indivíduos originalmente recrutados para o ADNI-1 e ADNI-GO tinham a opção de serem seguidos no ADNI-2.

4.3.1 Aspectos Éticos

O estudo ADNI foi aprovado pelos comitês de ética e pesquisa de cada centro participante e foi realizado em conformidade com os regulamentos federais, e as recomendações da Internal Conference on Harmonization (ICH) and Good Clinical Practices (GCP). Os participantes do estudo forneceram consentimento informado por escrito no momento do recrutamento e preencheram os questionários que foram aprovados pelas comissões de ética de cada centro participante.

A base de dados ADNI é de livre acesso e foi disponibilizada para realização deste trabalho na McGill University, onde parte do trabalho foi executado. A realização do presente estudo foi aprovada pela Comissão Científica e pelo Comitê de Ética da PUCRS, conforme documentos anexos nesta tese.

Maiores informações sobre o estudo e seus protocolos estão disponíveis no site www.adni-info.org .

4.3.2 Amostra do estudo

Os critérios utilizados para seleção de indivíduos nos bancos de dados ADNI-GO e ADNI-2 foram: idade entre 55 e 90 anos, disponibilidade dos escores de MEEM e CDR e realização de protocolo completo de RMN incluindo DTI e PET cerebral com [^{18}F]Florbetapir e [^{18}F]FDG.

Os indivíduos selecionados para essa pesquisa foram classificados como cognitivamente normais (CN, N = 27), com comprometimento cognitivo leve (CCL, N = 49) e com Doença de Alzheimer (DA, N = 20).

4.4 PROTOCOLOS DE IMAGEM

4.4.1 Ressonância magnética nuclear com Imagem por Tensor de Difusão (DTI)

Todas as imagens de difusão foram adquiridas em aparelhos de 3 tesla da GE Medical Systems (GE), utilizando 41 direções do gradiente de codificação de difusão com b=1000 s/mm2 e 5 aquisições utilizando b=0 s/mm2, com tamanho de voxel de 1,4 milímetros x 1,4 milímetros x 2,7 milímetros. Todos os exames utilizados já estavam corrigidos no formato EPI-eddy pelo ADNI. As imagens em T1 foram adquiridas no mesmo aparelho de 3 tesla da GE, com um tamanho e voxel de 1,2 milímetros x 1,0 milímetro x 1,0 milímetro. Mais detalhes sobre o protocolo de aquisição estão disponíveis no site ADNI (ADNI-INFO.org).

Após o processamento das imagens de DTI, mapas de FA foram gerados usando FSL-DTIFIT a partir das imagens corrigidas no espaço nativo da RMN. Foi realizada uma análise para comparar cada grupo (CN, CCL, AD) em relação a valores de FA. Na sequencia, foi realizada uma análise através do contraste entre os grupos, mapeando apenas diferenças significativas entre os grupos CN vs DA **(Figura 9)**.

O fascículo angular foi definido como uma região de interesse para as análises subsequentes, uma vez que é uma área intimamente relacionada com a patologia da

DA, conectando o hipocampo ao córtex entorrinal. Através de imagens tridimensionais, o autor delimitou manualmente uma máscara com apenas um voxel de interesse (VOI) bilateralmente no fascículo angular, a fim de obter o valor da FA de cada sujeito **(Figura 10)**.

Figura 9. Sumário dos métodos de imagem utilizados no protocolo do presente estudo.

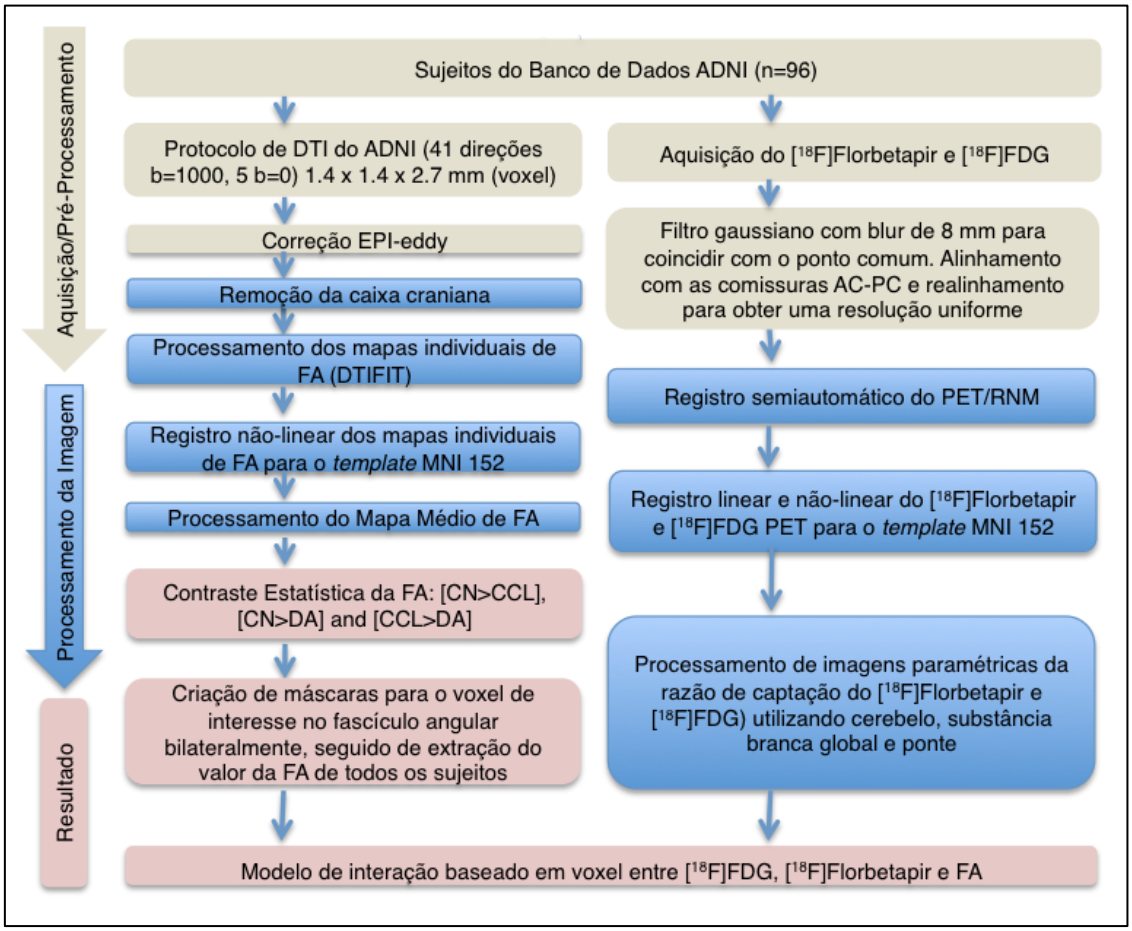
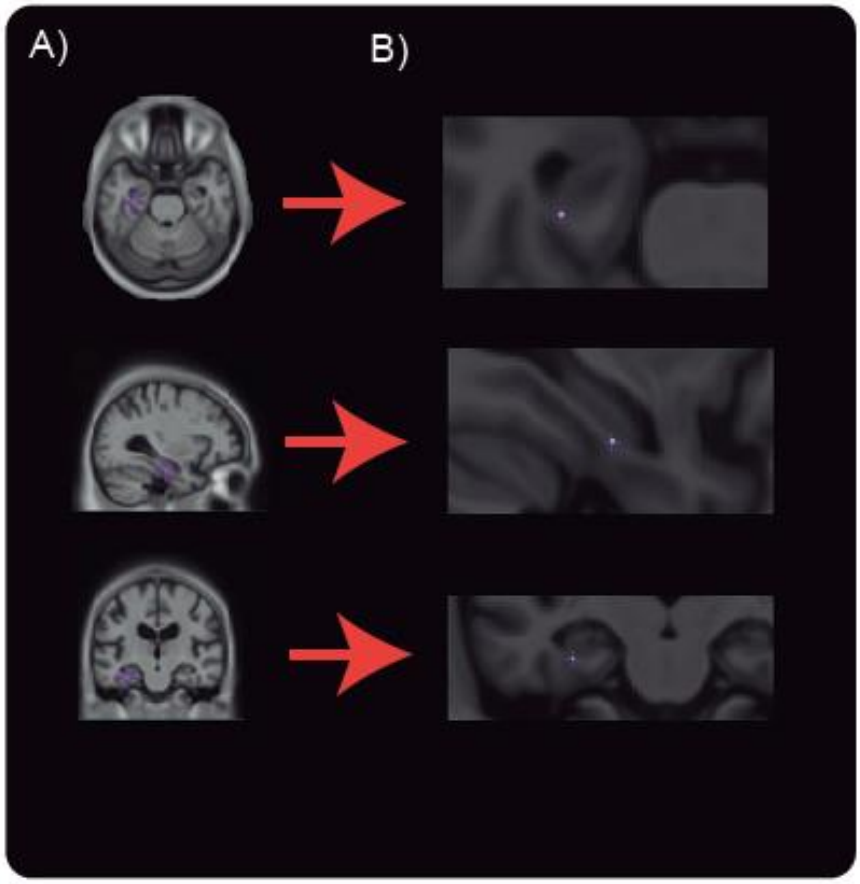


Figura 10. Ilustração representando o processo para obter uma máscara para o voxel de interesse no fascículo angular esquerdo em uma imagem T1 de RMN. Através dessa máscara, são obtidos os valores da FA neste voxel em todos os pacientes.



4.4.2 Tomografia por Emissão de Pósitrons

As imagens de PET adquiridas para a análise já foram submetidas a um ‘’blur’’ para coincidir com uma função de propagação do ponto comum semi-máxima com 8 milímetros de largura total. As imagens também foram alinhadas com as comissuras anterior e posterior e realinhadas para obter uma resolução uniforme comum. Subsequentemente, as imagens de PET foram submetidas a normalização espacial não-linear para o espaço do *template* MNI 152, utilizando a transformação derivada da transformação de PET / T1-RM semi-automática e registro de ressonância magnética anatômica para cada sujeito.

Os mapas com valores de *standardized uptake value ratio* (SUVR) baseado em voxel, foram então gerados para [^{18}F]Florbetapir usando como pontos de referência a substância cinzenta do cerebelo e a substância branca global. As imagens do SUVR de [^{18}F] FDG foram geradas utilizando a ponte como a região de referência.

Um valor global da SUVR para cada sujeito foi estimado utilizando uma ‘’máscara’’ do cérebro incluindo os córtices pré-frontal, orbito-frontal, parietal, temporal, cíngulo anterior e posterior e pré-cúneo.

Os protocolos de aquisição padrão de PET são detalhados no seguinte endereço eletrônico: http://adni.loni.usc.edu/methods.

4.5 MÉTODOS ESTATÍSTICOS

As análises estatísticas foram realizadas utilizando o software R Statistical Software Package versão 3.0.2 com a biblioteca RMINC (http://www.r-project.org/). RMINC (https://wiki.mouseimaging.ca/display/MICePub/RMINC) é um pacote de imagem que permite que os arquivos de imagem no formato de imagens médicas NetCDF (MINC) sejam analisados dentro do ambiente estatístico R.

4.5.1 Análise da FA

Na primeira parte de nossa análise, através de análise de covariância (ANCOVA), comparamos a FA em diversas áreas entre os grupos. Na comparação CN vs DA, no grupo DA identificamos *clusters* significativos de redução da FA no fascículo angular bilateral e no fórnix ($P<0.05$). A FA no voxel com maior diferença estatística no fascículo angular foi extraída bilateralmente através de uma máscara delimitada manualmente no voxel de interesse (VOI).

Na análise de co-variância (ANCOVA), foram consideradas covariáveis idade, gênero, educação, status APOE $\varepsilon 4$ e escore de Hachinski.

4.5.2 Comparações entre os grupos

Foram realizadas comparações entre os grupos (CN vs CCL, CN vs DA, CCL vs DA) em relação à FA no voxel de interesse e SUVR global no PET [^{18}F]Florbetapir e no PET [^{18}F]FDG.

A análise de co-variância (ANCOVA) foi aplicada em todas as comparações, sendo consideradas covariáveis idade, gênero, educação, status APOE $\varepsilon 4$ e escore de Hachinski.

4.5.3 Análise da interação entre FA e deposição de amiloide

Para avaliar os efeitos das anormalidades focais na substância branca (FA no VOI do fascículo angular bilateral), do depósito regional de β-amiloide ([^{18}F]Florbetapir SUVR nas regiões de interesse) e da interação entre ambos no hipometabolismo regional de glicose ([^{18}F]FDG SUVR nas regiões de interesse), utilizamos o seguinte modelo estatístico:

$$[^{18}F]FDG\ SUVR \sim [^{18}F]\ Florbetapir\ SUVR + FA_v + FA_v * [^{18}F]\ Florbetapir\ SUVR + covariáveis + erro$$

A análise referente ao índice de FA no VOI no fascículo angular bilateral e ao PET SUVR foi realizada utilizando o software VoxelStats (79). O software VoxelStats foi especialmente criado para fazer a análise aqui apresentada. VoxelStats tem a capacidade de utilizar modelos estatísticos a cada voxel, de forma a determinar os efeitos estatísticos de cada um dos fatores, bem como os das respectivas interações.

O escore de Hachinski (80) foi utilizado para limitar o impacto de possíveis lesões vasculares nas alterações da substância branca, e também idade, gênero, educação e status APOE ε 4 foram incluídos como covariáveis.

Os mapas estatísticos paramétricos foram corrigidos por múltiplas comparações utilizando um $P<0.05$ (81).

5 RESULTADOS

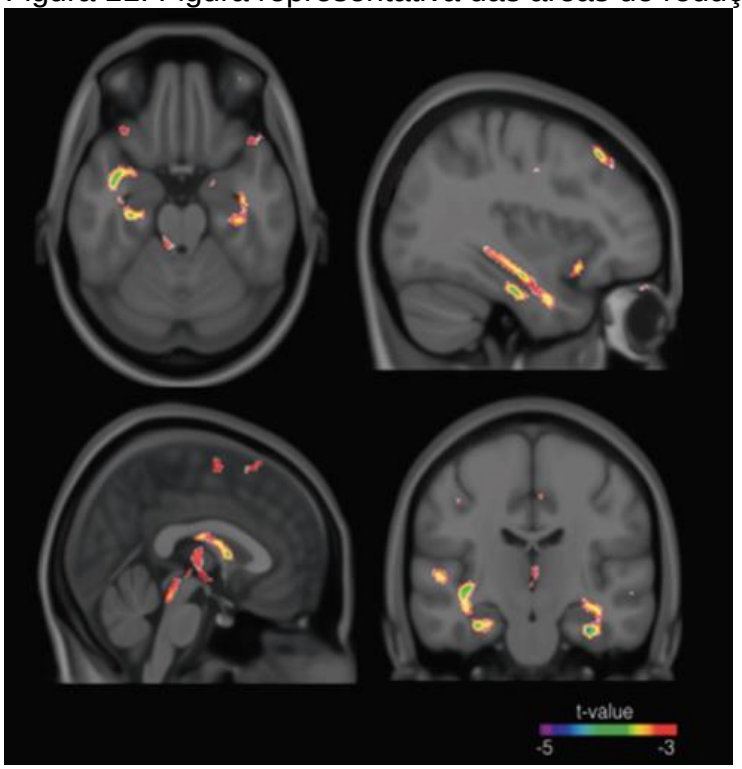
5.1 DIFERENÇAS ENTRE OS MAPAS DE FA

O primeiro dado a ser apresentado é o mapa das diferenças na FA entre os grupos, uma vez que foi o passo fundamental para a seleção do voxel de interesse utilizado nas análises subsequentes. Resultados adicionais relativos à FA, especificamente relacionados ao VOI, estão descritos mais adiante.

Na comparação dos mapas médios de FA entre os grupos CN e DA, foi observada uma redução nos índices de FA na substância branca parahipocampal, incluindo o fascículo angular bilateral e o fórnix no grupo DA (P<0.05) **(Figura 11).**

Não foram observadas diferenças estatisticamente significativas entre os grupos CN vs CCL e CCL vs DA.

Figura 11. Figura representativa das áreas de redução de FA no grupo DA.



As áreas de redução de FA nos pacientes com DA (n=20) comparados com os CN (n=27) estão ilustrados em cortes axial, sagital e coronal. Os voxels de redução significativa da FA estão sobrepostos a uma imagem de RMN em T1, representativa no espaço esterotáxico. A análise estatística evidenciou redução na FA na substância branca parahipocampal, fascículo angular e fórnix (P<0.05, corrigidos para idade, gênero, educação, escore de Hachinski e status APOE ε4). Entre outras áreas, a figura ressalta a redução da microestrutura (FA) do fascículo angular bilateralmente.

5.2 DADOS DEMOGRÁFICOS E DE INDÍCES DOS BIOMARCADORES

Os dados demográficos, os valores da FA no voxel de interesse (VOI) do fascículo angular bilateral e o SUVR global do PET [^{18}F]Florbetapir e PET [^{18}F] FDG de todos os pacientes estão expressos na **Tabela 1**.

Os valores médios e o desvio padrão dos índices demográficos e dos biomarcadores em cada grupo estão expressos na **Tabela 2.**

Tabela 1. Dados demográficos e valores dos biomarcadores de todos os pacientes

ID	Diagnóstico	Idade	Genêro	APOE	Educação	MEEM	Hachinski	CDR	[^{18}F]FDG SUVR global	[^{18}F]Florbetapir SUVR global	FA - VOI Esquerda	FA - VOI Direita
4003	CN	74.5	F	1	16	30	1	0	1.082711123	1.191738006	0.169231358	0.182676122
4050	CN	77.3	M	0	19	24	1	0	1.114448705	0.9488903	0.218049821	0.195766456
4076	CN	72.7	F	0	20	30	0	0	1.332052698	1.000132634	0.10099514	0.15533819
4081	CN	73	F	1	12	28	1	0	1.085424355	1.292955925	0.136289537	0.139499108
4086	CN	82	M	0	20	28	1	0	1.203456996	0.955336791	0.121016414	0.171692137
4104	CN	72.6	M	0	20	30	1	0	1.289989798	1.057865339	0.114283892	0.162085125
4119	CN	79.5	M	0	20	30	1	0	1.161890609	0.907200694	0.149801426	0.189997995
4121	CN	89.2	M	0	16	27	1	0	1.004391883	0.947830818	0.194020249	0.230955995
4198	CN	78.5	F	1	16	30	1	0	1.196606261	1.392925997	0.075187366	0.132193611
4234	CN	72.3	M	1	13	28	1	0	1.212103988	1.032014789	0.178989428	0.171611724
4290	CN	73.8	M	1	20	29	0	0	1.102500143	1.2902265	0.152017662	0.136573373
4371	CN	67.9	M	1	18	28	1	0	1.174035608	1.245840307	0.102273634	0.203078472
4385	CN	68.7	F	1	12	30	0	0	1.154065601	1.43819295	0.130428663	0.105598655
4441	CN	68.9	F	0	16	30	1	0	1.423428244	1.058664756	0.126106845	0.168409712
4499	CN	84	M	0	20	28	1	0	1.151958835	0.955943957	0.152009722	0.177644184
4503	CN	73.9	F	0	19	30	0	0	1.10712224	0.974887379	0.155547227	0.165571851
4585	CN	65.7	M	1	13	29	1	0	1.068407891	1.000992323	0.130986782	0.158363785
4604	CN	65.1	M	1	19	29	1	0	1.290338752	0.973852217	0.151228804	0.113883163
4620	CN	77.3	M	0	19	30	1	0	1.23153927	1.142586445	0.128342491	0.139953306
4637	CN	71	F	0	18	29	0	0	1.373128952	1.124544236	0.092626725	0.150715964
4638	CN	74.3	M	0	17	28	1	0	1.286969422	0.981856412	0.141875187	0.178856612
4645	CN	78.5	F	0	14	30	1	0	1.127338268	1.089717841	0.133404642	0.151082921
4649	CN	65.6	M	0	18	30	0	0	1.338425231	1.069980378	0.169838904	0.145529518
4652	CN	79.6	M	0	20	26	1	0	1.060286561	1.34135711	0.214804039	0.188950904

Tabela 1. Dados demográficos e valores dos biomarcadores de todos os pacientes. (cont.)

ID	Diagnóstico	Idade	Sexo	APOE4	Educação	MEEM	Hachinski	CDR	[^{18}F]FDG SUVR global	[^{18}F]Florbetapir SUVR global	FA - VOI Esquerda	FA - VOI Direita
4872	CN	68.7	F	1	14	26	0	0	1.389058617	1.203588163	0.152583557	0.13483449
4952	CN	69.6	F	1	12	28	1	0	1.299009388	1.008282068	0.140357589	0.129214191
5236	CN	85.5	M	0	16	28	0	0	1.157056493	1.10794248	0.209346685	0.182411296
2031	CCL	72.8	M	0	16	27	1	0.5	1.382243963	1.047213274	0.155268203	0.116309799
2047	CCL	77.6	M	1	18	27	3	0.5	1.229617779	1.370946139	0.205408031	0.202200026
2052	CCL	73.3	M	0	20	29	2	0.5	1.28591816	0.956417506	0.115494981	0.214422288
2079	CCL	65.7	M	0	13	25	0	0.5	1.240553029	1.327601423	0.141883146	0.146100142
2106	CCL	77.7	M	0	12	29	1	0.5	1.151474751	1.360098609	0.157059333	0.090771018
2146	CCL	68.5	M	0	18	29	1	0.5	1.454927956	1.075635414	0.075992541	0.107470749
2200	CCL	76.3	F	0	14	26	3	0.5	1.142191236	0.975792403	0.079799827	0.138745068
2216	CCL	68.3	M	1	14	27	0	0.5	1.111077085	1.223608499	0.180267991	0.174114367
2284	CCL	76.8	M	1	16	29	1	0.5	1.14680193	1.157166799	0.196214756	0.149745869
2332	CCL	70.7	F	0	16	28	1	0.5	1.351879219	0.998777138	0.126099315	0.164961847
2336	CCL	75.2	F	1	12	30	1	0.5	1.19632517	1.457959488	0.22332364	0.14204803
2367	CCL	74.9	M	1	20	25	1	0.5	1.436256792	1.1433128	0.148167929	0.156598782
2374	CCL	81.4	F	0	18	26	0	0.5	1.163611437	0.889229319	0.242276345	0.135669172
2376	CCL	82.3	M	1	17	28	1	0.5	1.061494987	1.369676554	0.108380007	0.203811164
2390	CCL	88	F	0	12	25	0	0.5	1.229797388	1.331233077	0.102623683	0.082465178
2394	CCL	69.6	M	1	20	30	0	0.5	1.364075347	1.047884254	0.087412468	0.099183402
2395	CCL	73	M	1	19	30	0	0.5	1.119787175	1.164176989	0.170783016	0.325910473
2398	CCL	73.7	M	1	13	29	0	0.5	1.203207341	1.079778727	0.107424668	0.130360915
4157	CCL	81.4	F	0	19	29	1	0.5	1.243049549	1.357271189	0.079442841	0.135568467
4162	CCL	71.5	F	1	16	26	0	0.5	1.075877375	1.351042883	0.159135269	0.113619486
4168	CCL	82.3	M	1	17	29	1	0.5	1.120698704	0.985955052	0.114235918	0.106844601

Tabela 1. Dados demográficos e valores dos biomarcadores de todos os pacientes. (cont.)

ID	Diagnóstico	Idade	Sexo	APOE4	Educação	MEEM	Hachinski	CDR	[^{18}F]FDG SUVR global	[^{18}F]Florbetapir SUVR global	FA - VOI Esquerda	FA - VOI Direita
4197	CCL	81.5	M	1	20	30	1	0.5	1.0491397	1.202289935	0.143728228	0.123826355
4205	CCL	81.7	F	0	18	29	0	0.5	1.187506994	1.293678029	0.11982314	0.157265118
4210	CCL	64.1	M	1	18	29	0	0.5	1.431606802	1.03240484	0.182266538	0.153844308
4220	CCL	71.4	F	0	18	30	1	0.5	1.059830382	1.122166145	0.206168708	0.122756254
4245	CCL	73.8	M	1	19	26	1	0.5	1.129526477	1.039625755	0.090902628	0.080064543
4272	CCL	71	M	0	12	28	1	0.5	1.234733366	1.130374992	0.059653839	0.085406611
4287	CCL	71	F	1	17	29	1	0.5	1.222140775	1.339936361	0.103603813	0.066433637
4301	CCL	74.7	M	0	18	28	2	0.5	1.086579523	0.99892808	0.063052626	0.083791589
4455	CCL	64.4	M	0	18	28	1	0.5	1.085364148	0.959431837	0.174516732	0.152501247
4584	CCL	78.5	F	1	16	27	0	0.5	1.20325766	1.202100199	0.163915477	0.124879536
4624	CCL	77.9	F	1	13	30	1	0.5	1.150905523	1.30006599	0.108872187	0.150180206
4626	CCL	69.3	M	0	18	29	1	0.5	1.191975651	0.996051695	0.092178841	0.088574582
4646	CCL	61	F	1	16	30	0	0.5	1.310626006	1.180296619	0.22043882	0.227838183
4712	CCL	74.5	M	1	17	24	1	0.5	1.222456408	1.352214297	0.078564813	0.205189866
4765	CCL	76.1	M	1	16	26	1	0.5	1.056968922	1.139159062	0.14026882	0.11782578
4807	CCL	72.2	F	1	16	29	0	0.5	1.328401718	1.443104406	0.140668856	0.128060805
4857	CCL	68.5	M	1	16	27	1	0.5	1.04811218	1.482732664	0.109955641	0.193410073
4858	CCL	55.4	M	1	16	30	0	0.5	1.299659254	1.261780968	0.137826853	0.19641479
4869	CCL	77.5	M	0	19	29	0	0.5	1.2858183	0.983220448	0.078060144	0.109477608
4885	CCL	75.1	M	1	20	29	0	0.5	1.158162037	1.225388056	0.159424648	0.081828113
4888	CCL	74.9	M	1	12	25	1	0.5	1.137832986	1.300259935	0.132093128	0.119092555
4897	CCL	76	F	0	11	29	1	0.5	1.115290235	1.311881356	0.121865253	0.194858595
4902	CCL	75.5	F	1	15	25	1	0.5	1.154561484	1.267353516	0.099422808	0.073882251
4926	CCL	62.6	M	1	18	29	0	0.5	1.289455034	1.07599507	0.107337534	0.195945265

Tabela 1. Dados demográficos e valores dos biomarcadores de todos os pacientes. (cont.)

ID	Diagnóstico	Idade	Sexo	APOE4	Educação	MEEM	Hachinski	CDR	[^{18}F]FDG SUVR global	[^{18}F]Florbetapir SUVR global	FA - VOI Esquerda	FA - VOI Direita
4928	CCL	78	M	1	16	27	1	0.5	1.185666722	1.356936489	0.086268583	0.135516501
4944	CCL	68.3	M	1	14	29	0	0.5	1.316023496	1.188394005	0.174596196	0.151515135
4945	CCL	56.8	M	1	12	27	0	0.5	1.101368756	1.24324712	0.147648397	0.104419653
5007	CCL	72	M	0	14	27	1	0.5	1.211150055	1.313963207	0.180867463	0.190273167
4136	DA	66.9	M	1	20	24	0	1	1.066845744	1.461146822	0.130672927	0.116630165
4215	DA	81.9	M	1	20	26	1	0.5	1.163813043	1.538081223	0.102695423	0.10625056
4307	DA	79.3	M	1	16	22	0	0.5	0.945371848	1.244014931	0.110798293	0.136288719
4591	DA	66.1	F	1	13	23	0	1	1.359621617	1.430098149	0.146973689	0.150249853
4707	DA	68.1	M	1	14	21	1	1	1.106052629	1.25368723	0.125178552	0.13963948
4718	DA	78.7	M	1	12	20	1	1	0.94649303	1.440908864	0.10507485	0.181506299
4892	DA	75.3	F	1	11	24	1	1	1.206239894	1.361071823	0.155640736	0.136197652
4924	DA	77.5	M	0	14	20	4	1	1.061825814	1.128983175	0.123025344	0.128110102
4959	DA	77.7	M	1	20	25	1	1	1.146265484	1.41287499	0.104934386	0.072897631
4962	DA	80.3	F	1	18	22	1	0.5	0.8500002	1.252130993	0.125009124	0.129563237
4964	DA	81.1	M	0	16	23	0	1	1.017801616	1.497713869	0.105605191	0.080797166
4992	DA	63.9	F	1	16	22	1	1	1.087821094	1.488781489	0.089809935	0.105860542
5038	DA	81.8	M	0	18	25	0	1	1.034791984	1.024873424	0.144520207	0.11843339
5056	DA	85.5	M	1	20	20	0	1	0.987296584	1.278697004	0.119616831	0.099211021
5057	DA	75.6	M	0	16	26	1	0.5	1.323604624	0.947070269	0.133998602	0.140853237
5058	DA	61.9	M	0	20	26	0	0.5	1.214164622	1.523428363	0.093022826	0.073069654
5062	DA	71.3	F	1	14	26	0	0.5	1.296496008	1.409666454	0.155284191	0.157574452
5165	DA	79	M	0	12	23	1	1	1.00420936	1.355500074	0.123003617	0.121143037
5196	DA	73	F	1	18	21	0	0.5	1.183457581	1.371473022	0.093875445	0.137302781
5251	DA	66.5	F	1	16	26	1	0.5	1.309879062	2.134269373	0.158043031	0.113811765

Legenda: Idade expressa em anos; Sexo, F= Feminino, M= Masculino; APOE4, 1= ao menos um alelo APOE ε 4 positivo, 0 = negativo; Educação expressa em anos, Mini-Exame do Estado Mental (MEEM) e escala de Hachinski expresso em escore total.

Tabela 2. Média dos dados demográficos e dos biomarcadores em cada grupo.

Características	Total	CN	CCL	DA
No.	96	27	49	20
Idade, anos, média (DP)	73.81 (6.51)	74.4 (6.2)	73.1 (6.5)	74.5 (6.9)
Sexo Masculino, No. (%)	62 (64)	16 (59)	33 (67)	13 (65)
APOE ε 4 positivo, No. (%)	55 (57)	11 (40)	30 (61)	14 (70)
Educação, anos, média (DP)	16.39 (2.74)	16.9 (2.8)	16.1 (2.5)	16.2 (2.9)
MEEM, escore, média (DP)	27.14 (2.68)	28.6 (1.5)	27.9 (1.6)	23.2 (2.1)
Hachinski, escore, média (DP)	0.71 (0.70)	0.70 (0.46)	0.73 (0.72)	0.7 (0.92)
CDR, escore, média (DP)	0.42 (0.31)	0 (0)	0.5 (0)	0.8 (0.25)
[^{18}F]FDG, SUVR global, média (DP)	1.18 (0.12)	1.20 (0.11)	1.20 (0.10)	1.11 (0.14)
[^{18}F]Florbetapir, SUVR global, média (DP)	1.20 (0.19)	1.10 (0.14)	1.19 (0.15)	1.37 (0.23)
FA VOI Esquerda, média (DP)	0.13 (0.03)	0.14 (0.03)	0.13 (0.04)	0.12 (0.02)
FA VOI Direita, média (DP)	0.14 (0.04)	0.16 (0.02)	0.14 (0.04)	0.12 (0.02)

5.2.1 Diferenças de FA no Voxel de Interesse (VOI) Entre os Grupos

Os grupos apresentaram valores médios distintos em relação à FA- VOI no fascículo angular bilateralmente. Através de ANCOVA, foi calculada a significância das análises incluindo idade, gênero, educação, escore de Hachinski e status APOE ε 4 como covariáveis.

Foi observada diferença significativamente estatística apenas na comparação entre os grupos CN e DA na FA-VOI à direita (**Tabela 4)**.

Tabela 3. FA- VOI no Fascículo Angular Esquerdo.

Análise entre os grupos	*P*
CN vs CCL	0.45
CN vs DA	0.1
CCL vs DA	0.46

Tabela 4. FA- VOI no Fascículo Angular Direito.

Análise entre os grupos	*P*
CN vs CCL	0.11
CN vs DA	0.01
CCL vs DA	0.19

5.2.2 Diferenças nos Valores de PET [^{18}F]Florbetapir entre os Grupos

Os grupos apresentaram valores médios distintos em relação aos valores de PET [^{18}F]Florbetapir global SUVR. Através de ANCOVA, foi calculada a significância das análises incluindo idade, gênero, educação, escore de Hachinski e status APOE ε 4 como covariáveis.

Foi observada diferença significativamente estatística nas comparações entre os grupos CN vs DA e CCL vs DA **(Tabela 5)**.

Tabela 5. Comparação Entre os Grupos CN vs DA e CCL vs DA (PET [^{18}F]Florbetapir SUVR global).

Análise entre os grupos	*P*
CN vs CCL	0.2
CN vs DA	0.01
CCL vs DA	0.01

5.2.3 Diferenças nos Valores de PET [^{18}F]FDG entre os Grupos

Os grupos apresentaram valores médios distintos em relação aos valores de PET [^{18}F]FDG global SUVR. Através de ANCOVA, foi calculada a significância das análises incluindo idade, gênero, educação, escore de Hachinski e status APOE ε 4 como covariáveis.

Foi observada diferença significativamente estatística na comparação entre os grupos CCL vs DA **(Tabela 6)**.

Tabela 6. Comparação Entre os Grupos CN vs DA e CCL vs DA (PET [^{18}F]FDG SUVR global).

Análise entre os grupos	*P*
CN vs CCL	0.97
CN vs DA	0.08
CCL vs DA	0.02

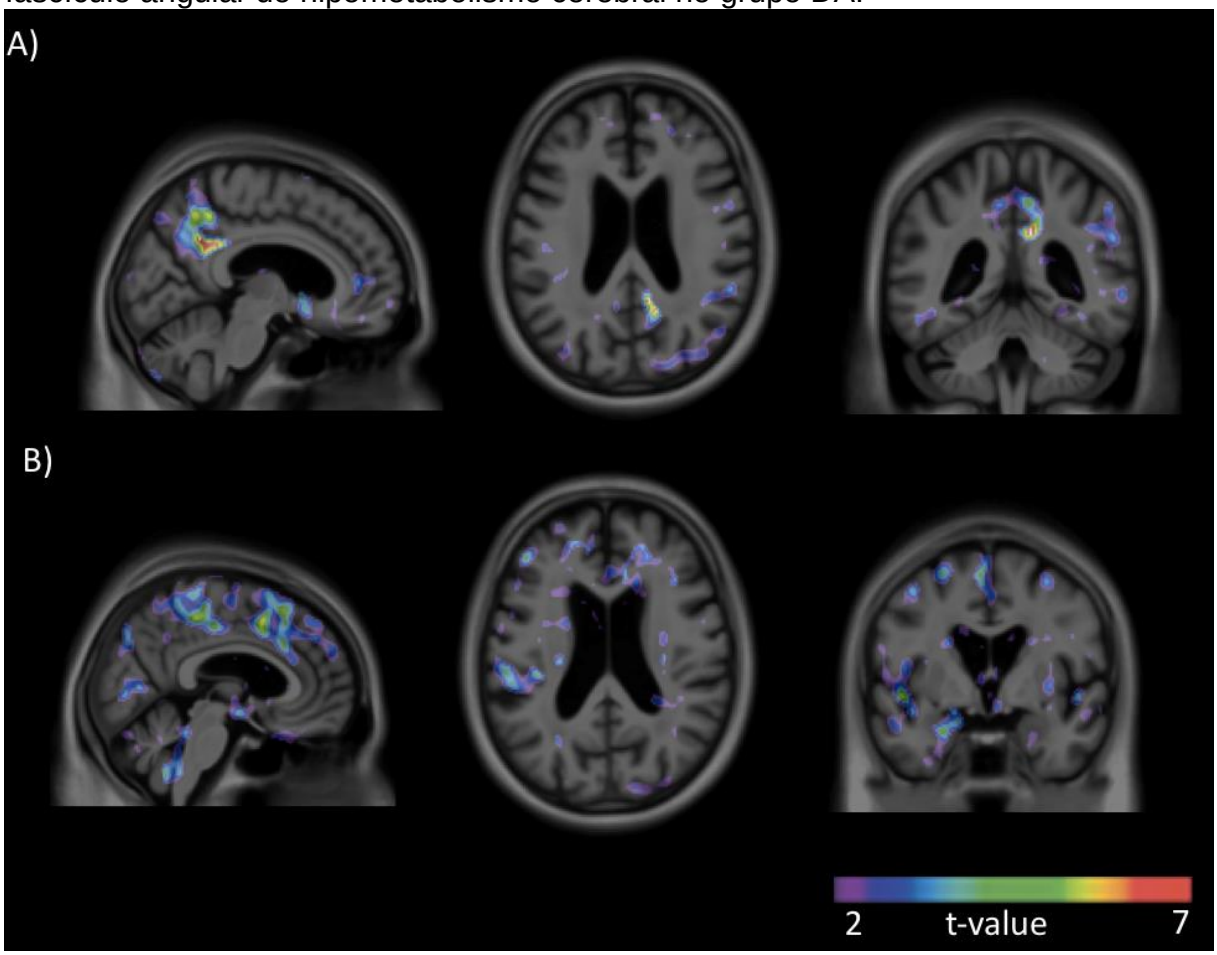
5.2.4 Efeito da Interação da Redução da FA no Metabolismo Cerebral

No grupo DA, a análise estatística revelou que a redução de FA no voxel de interesse no fascículo angular bilateral está associada com o hipometabolismo no

PET [^{18}F]FDG. O efeito da interação foi observada no corpo estriado, córtex orbito-frontal, basal e mesial temporal, pré-cúneo e cíngulo anterior e posterior **(Figura 12)**.

Não foram encontrados resultados significativos nesse modelo nos outros grupos analisados.

Figura 12. Figura representativa do efeito da interação da redução da FA no fascículo angular do hipometabolismo cerebral no grupo DA.



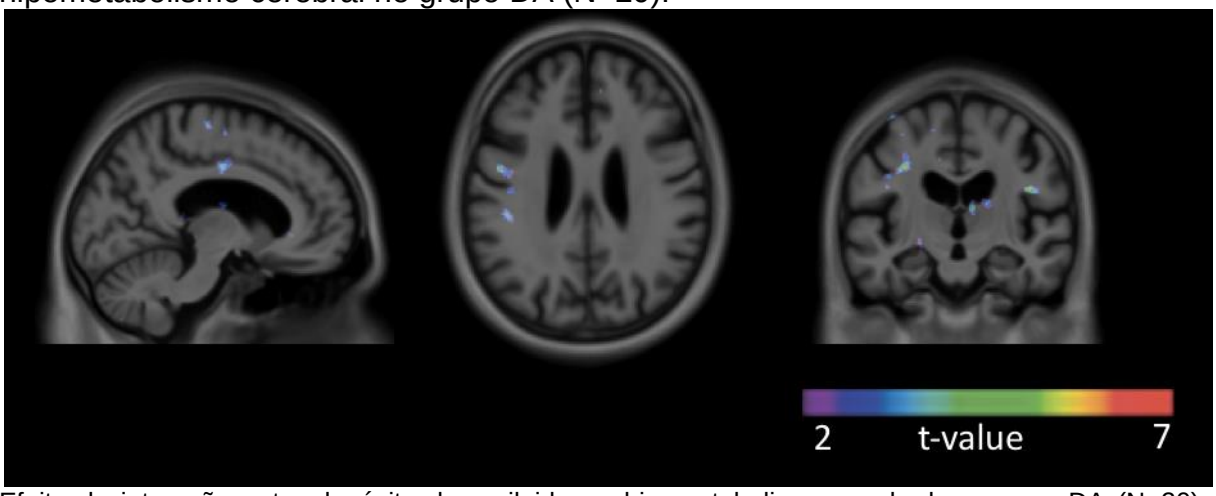
A) mostra o efeito da redução da FA no fascículo angular direito no hipometabolismo cerebral no grupo DA (N=20); (B) mostra o efeito da redução da FA no fascículo angular esquerdo no hipometabolismo cerebral no grupo DA (N=20). Mapas estatísticos paramétricos após correção por múltiplas comparações (P<0.05), sobrepostos em uma imagem de RMN em T1, representativa no espaço esterotáxico, revelaram áreas de redução de PET [^{18}F]FDG como uma função da FA no VOI do fascículo angular em cortes coronal, axial e sagital. Interações significativas foram observadas no pré-cúneo, cíngulo e córtex temporo-parietal. A análise foi corrigida para idade, gênero, educação, escore de Hachinksi e status APOE ε4.

5.2.5 Efeito da Interação do Depósito de Amiloide no Hipometabolismo Cerebral

No grupo DA, a análise estatística revelou que a interação entre o aumento do PET [^{18}F] Florbetapir SUVR nas regiões de interesse está associada com o hipometabolismo no PET [^{18}F]FDG. O efeito da interação foi observada no corpo estriado, cíngulo e córtex temporo-parietal **(Figura 13)**.

Não foram encontrados resultados significativos nesse modelo nos outros grupos analisados.

Figura 13. Figura representativa do efeito da interação entre depósito de amiloide no hipometabolismo cerebral no grupo DA (N=20).



Efeito da interação entre depósito de amiloide no hipometabolismo cerebral no grupo DA (N=20). Mapas estatísticos paramétricos após correção por múltiplas comparações (P<0.05), sobrepostos em uma imagem de RMN em T1, representativa no espaço esterotáxico, revelaram áreas de redução de PET [^{18}F]FDG como uma função do PET [^{18}F] Florbetapir SUVR em cortes coronal, axial e sagital. Interações significativas foram observadas no corpo estriado, cíngulo e córtex temporo-parietal. A análise foi corrigida para idade, gênero, educação, escore de Hachinksi e status APOE ε 4.

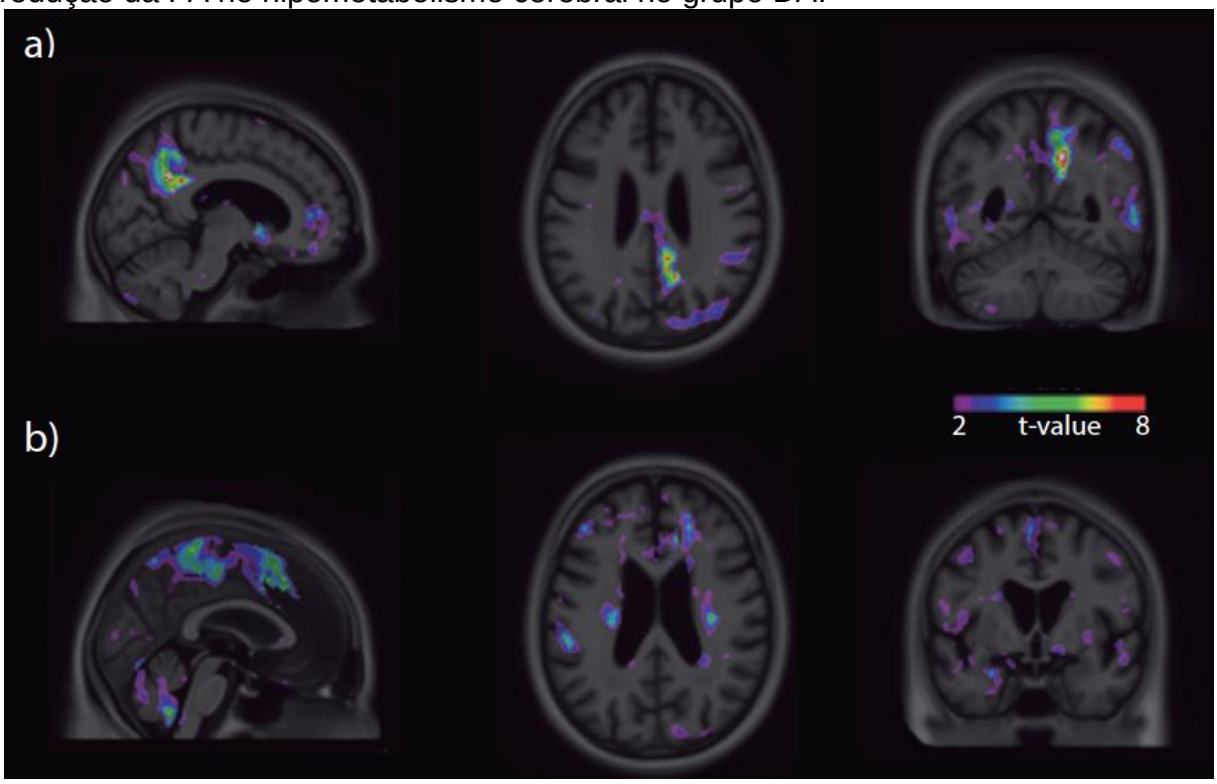
5.2.6 Efeito da Interação entre a Redução da FA e o PET [^{18}F]Florbetapir no Hipometabolismo Cerebral

No grupo DA, a análise estatística revelou que a interação entre a redução na FA no voxel de interesse no fascículo angular bilateral e o aumento do PET [^{18}F] Florbetapir SUVR nas regiões de interesse está associada com o hipometabolismo no PET [^{18}F]FDG. O efeito da interação foi observado no corpo estriado, córtex orbito-frontal, basal e mesial temporal, pré-cúneo e cíngulo anterior e posterior **(Figura 14).**

As áreas afetadas foram distintas dependendo do lado em que a desconexão no fascículo angular foi analisada. A interação entre a desconexão no fascículo angular direito e o depósito de amiloide foi associada com hipometabolismo no cíngulo posterior e pré-cúneo **(Figura 14 A),** enquanto alterações no fascículo angular esquerdo foram associados a um hipometabolismo no corpo estriado, córtex órbito-frontal, basal e mesial temporal e cíngulo anterior através desse modelo de interação **(Figura 14 B).**

Não foram encontrados resultados significativos nesse modelo nos outros grupos analisados.

Figura 14 . Figura representativa do efeito da interação entre depósito de amiloide e redução da FA no hipometabolismo cerebral no grupo DA.



A) Efeito da interação entre depósito de amiloide e FA no fascículo angular direito no hipometabolismo cerebral no grupo DA (N=20). B) Efeito da interação entre depósito de amiloide e FA no fascículo angular esquerdo no hipometabolismo cerebral no grupo DA (N=20). Mapas estatísticos paramétricos após correção por múltiplas comparações ($P<0.05$), sobrepostos em uma imagem de RMN em T1, representativa no espaço esterotáxico, revelaram áreas de redução de PET [^{18}F]FDG como uma função da interação entre PET [^{18}F] Florbetapir SUVR e FA no VOI em cortes coronal, axial e sagital. Interações significativas foram observadas no pré-cúneo, cíngulo posterior direito, corpo estriado esquerdo, córtex temporal mesial, órbito-frontal e cíngulo anterior esquerdo. A análise foi corrigida para idade, gênero, educação, escore de Hachinksi e status APOE ε 4.

6 DISCUSSÃO

A presente análise transversal de uma coorte de idosos com distintos status cognitivos mostrou que no grupo DA a interação entre o acúmulo de amiloide em diferentes áreas e a redução da FA no fascículo angular associou-se significativamente com hipometabolismo em regiões límbicas. Observamos também que a desconexão do fascículo angular apresenta um efeito individual prepoderante, quando comparado com o depósito de amiloide, para o hipometabolismo cerebral. Estes achados contribuem para um conceito integrativo da fisiopatologia da DA, sugerindo que a concomitância de diferentes processos patológicos impacta no metabolismo cerebral e, consequentemente, na progressão da doença.

Inicialmente, como observado na literatura, confirmamos a observação de que a FA em feixes de substância branca associados ao sistema de memória, incluindo o fascículo angular e o fornix, apresenta redução em pacientes com DA em comparação com controles CN (82-84). Alterações microestruturais nesses feixes comprometem conexões entre áreas corticais, levando a distintos níveis de "isolamento" ou deaferentação (85). A maioria das alterações foram evidenciadas nos lobos temporais, regiões descritas no modelo da retrogênese como especialmente suscetíveis ao processo degenerativo axonal na DA (47, 86, 87). Por outro lado, em contraste com outros estudos e possivelmente devido ao limitado número de indivíduos selecionados para esta pesquisa, não encontramos diferenças significativas entre os grupos CN vs CCL e CCL vs DA (71, 88, 89).

Estudos prévios avaliaram as relações entre diferentes biomarcadores de DA, demonstrando correlação entre redução da FA e tanto hipometabolismo cerebral regional (73) quanto atrofia hipocampal (90). Nossos achados estão de acordo com estes resultados, demonstrando hipometabolismo cerebral associado a desconexão do fascículo angular bilateral. Além disso, existem evidências de que o metabolismo no cíngulo posterior está inversamente relacionado com maior difusividade no hipocampo (91). Em conjunto, estes e outros estudos sugerem que atrofia hipocampal degenerativa leva à desconexão dos fascículos angular e do cíngulo, com subsequente hipometabolismo nessas áreas (92, 93). Por outro lado, não foram observadas associações entre comprometimento da substância branca e deposição de β-amiloide mensurada por PET nas fases pré-demência (90).

Em adultos cognitivamente normais, foram observadas associações entre biomarcadores no líquido cefalorraquidiano ($A\beta_{42}$ e $A\beta_{42}$/p-Tau_{181}) e integridade da

substância branca, particularmente correlação positiva entre $A\beta_{42}$/p-Tau_{181} e FA no fórnix, corpo caloso e fascículos inferior, superior e fronto-occipital inferior (94). Valores de FA significantemente mais baixos nas fibras do cingulum posterior esquerdo foram observadas em indivíduos com níveis patológicos de T-tau no líquido cefalorraquidiano (95), e maiores concentrações de $A\beta_{1-42}$ no líquido cefalorraquidiano foram diretamente correlacionadas com valores médios mais altos de FA (96). Cabe ressaltar que alterações da FA não ocorrem exclusivamente na DA, já tendo sido descritas em outras doenças neurodegenerativas, como Demência Frontotemporal e Demência com Corpos de Lewy (97, 98).

Em sujeitos assintomáticos com história familiar positiva para DA e em indivíduos cognitivamente normais, porém com PET Aβ +, já foram observados índices mais elevados de FA quando comparados com controles, sugerindo possíveis mecanismos compensatórios nas fases iniciais da doença, por possível resposta glial e ‘’pruning’’ (99, 100). Especulamos que estes mecanismos poderiam explicar, ao menos parcialmente, nossos resultados não significativos observados nos grupos CN e CCL. Estudos subsequentes com maior número de sujeitos assintomáticos e com CCL poderão elucidar essa questão.

Os valores de SUVR global do PET [^{18}F]FDG e [^{18}F]Florbetapir do nosso estudo foram semelhantes aos observados e propostos por outros autores (101, 102). Em relação ao PET [^{18}F]Florbetapir, observa-se um maior SUVR em pacientes com DA. O oposto ocorre em relação ao PET [^{18}F]FDG, uma vez que na DA há hipometabolismo cerebral, expresso por valores mais baixos de SUVR.

Através de nosso modelo de interação, observamos que, em indivíduos com DA, áreas de hipometabolismo associam-se concomitantemente à alterações no fascículo angular, evidenciada por menor FA no VOI, e ao depósito de β-amiloide, documentado através do aumento do SUVR de PET [^{18}F]Florbetapir nas regiões de interesse. O depósito de amiloide nas áreas em que encontramos hipometabolismo foi descrito por Braak nos estágios neuropatológicos B e C da progressão da DA, envolvendo áreas associativas no isocórtex de pacientes com declínio cognitivo significativo (22). Cabe ressaltar que o depósito de amiloide de forma isolada apresentou um efeito discreto no hipometabolismo cerebral, sugerindo que outros eventos patológicos, no caso a desconexão do fascículo angular, são fundamentais para a progressão da doença.

O advento dos biomarcadores para DA tem possibilitado a avaliação *in vivo* de diversos processos fisiopatológicos, e refinado a investigação e a caracterização ao

longo do *continuum* do envelhecimento normal e patológico. Os critérios diagnósticos clínicos vigentes para DA são baseados essencialmente no comprometimento clínico e funcional, porém os critérios de pesquisa já incorporam a utilização de biomarcadores para classificação do paciente ao longo do *continuum*, como no National Institute on Aging-Alzheimer's Association (NIA-AA), onde a positividade dos biomarcadores combinada com aspectos clínicos leva à classificação do paciente em um de três estágios: assintomático (DA pré-clínica), pré-demência (CCL devido a DA) ou demência (devido a DA) (3, 17, 103, 104). Do ponto de vista de aplicação clínica no contexto atual, os biomarcadores podem contribuir no diagnóstico de demências atípicas e de início precoce (60, 105).

Além da análise individual de biomarcadores, a integração de diferentes modalidades pode promover uma melhor compreensão dos mecanismos relacionados à progressão da DA. Por exemplo, foi observado que a carga de depósito de amiloide combinada aos níveis de proteína tau no líquido cefalorraquidiano e ao metabolismo cerebral basal contribuem para predizer a conversão de pacientes com CCL para demência (106-108). Estudos longitudinais com DTI conseguiram identificar alterações que precedem a progressão para estágios mais graves ao longo do *continuum* através da análise de feixes específicos de substância branca, particularmente aqueles relacionados ao sistema límbico (109-111). Estes avanços impulsionaram a constituição do *European DTI Study in Dementia (EDSD)*, um consórcio multicêntrico como características operacionais semelhantes ao ADNI, voltado para o estudo específico de pacientes ao longo do *continuum* na DA através dessa técnica (112). Iniciativas como o EDSD oferecem novas perspectivas na avaliação de riscos e da progressão, especialmente através de modelos que possam integrar informações de distintos biomarcadores.

Ao utilizarmos diferentes biomarcadores, buscamos identificar denominadores comuns. A identificação de áreas intimamente relacionadas à DA, como o cíngulo posterior e o pré-cúneo convergem com resultados de pesquisas realizadas por meio de métodos de neuroimagem distintos dos utilizados em nosso estudo, como ressonância magnética funcional (RMNf). A atividade cerebral padrão em repouso (em inglês *Default Mode Network - DMN*) avaliada através da RMNf é modulada por uma uma conexão em rede entre centros funcionais (113). Tanto o pré-cúneo quanto o córtex do cíngulo posterior desempenham um papel crucial na DMN, e estão relacionados com funções cognitivas como memória e atenção (114, 115). A identificação de hipometabolismo nessas regiões no nosso modelo de interação

contribui para o conceito de que essas áreas tem um função pivotal no desempenho cognitivo.

De forma específica, nossos resultados indicam também que a desconexão da substância branca tem um papel chave quando associada a depósitos de β-amiloide levando à progressão fisiopatológica na DA. Dessa forma, nossos achados indicam que a utilização de biomarcadores de integridade da substância branca pode auxiliar na identificação de quais pacientes com CCL e com depósitos de amiloide terão um maior risco de desenvolver demência.

Uma virtude metodológica da nossa análise é a utilização do programa VoxelStats (79), que tem a capacidade de utilizar modelos estatísticos a cada voxel, de forma a determinar os efeitos estatísticos de cada um dos fatores bem como os das respectivas interações. Quando esses dados são analisados de forma diferente (por exemplo, através da definição de toda uma região de interesse), o valor da FA ou do SUVR reflete uma média de todos os voxels na área selecionada, levando a uma possível subestimação dos achados. Outra característica importante da nossa pesquisa é o modelo desenvolvido especificamente com o objetivo de avaliar o impacto metabólico do efeito da interação entre alterações estruturais e depósito de amiloide em diferentes regiões cerebrais. Tais processos já foram avaliados de forma individual (73, 116, 117), mas nosso modelo é significativamente distinto ao propiciar uma análise integrativa desses dados. Consideramos também que a maior virtude metodológica é a utilização de variáveis contínuas. Os biomarcadores modificam-se de forma dinâmica em um *continuum* e, dessa forma, técnicas baseadas na dicotomização estão invariavelmente sujeitas a idiossincrasias analíticas e metodológicas.

Por outro lado, algumas questões metodológicas limitam a interpretação dos nossos resultados. Por tratar-se de estudo transversal, inferências sobre progressão do comprometimento da substância branca, do depósito de amiloide e do metabolismo de glicose são especulativas. Da mesma forma, como os critérios de inclusão exigiam a realização do protocolo completo de neuroimagem, incluímos um número relativamente pequeno de pacientes com DA. Estudos longitudinais e com um maior número de pacientes serão necessários para confirmação dos nossos achados.

Concluímos que na DA a interação entre depósito de β-amiloide e anormalidades em feixes da substância branca impactam de forma significativa o metabolismo cerebral em áreas relacionadas à cognição. A utilização de um modelo

estatístico integrando dados de diferentes biomarcadores contribuiu para podermos avaliar o efeito combinado de distintos processos patológicos na DA, gerando novas perspectivas científicas.

7 CONCLUSÕES

Concluímos que a magnitude do hipometabolismo nos pacientes com demência do tipo DA está associada à amiloidose e à desintegração das vias de projeções destas regiões. Através de um modelo que utiliza diferentes métodos de neuroimagem, concluímos que na DA a interação entre o aumento no SUVR do PET [^{18}F]Florbetapir nas regiões de interesse e a redução da FA no fascículo angular bilateral está associada com o declínio metabólico observado através de redução no SUVR do PET [^{18}F]FDG. Observamos também que a desconexão do fascículo angular apresenta efeito prepoderante, quando comparado com o depósito de amiloide, para o hipometabolismo cerebral.

A combinação de dados de metabolismo cerebral, de depósito de amiloide e da integridade dos feixes de substância branca que conectam importantes áreas cerebrais oferece a possibilidade de avaliar diferentes processos patológicos no mesmo paciente ao mesmo tempo. Este modelo traz a perspectiva de clarificar o papel dos distintos processos patológicos observados desde fases prodrômicas da DA e vai de encontro a uma teoria mais complexa e integrativa da sua fisiopatologia, sugerindo que é a combinação de diferentes processos patológicos que leva ao comprometimento metabólico cerebral.

REFERÊNCIAS

1. Oeppen J, Vaupel JW. Demography. Broken limits to life expectancy. Science. 2002;296(5570):1029-31.

2. Harman D. The free radical theory of aging. Antioxidants & redox signaling. 2003;5(5):557-61.

3. McKhann GM, Knopman DS, Chertkow H, Hyman BT, Jack CR, Jr., 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. Alzheimer's & dementia : the journal of the Alzheimer's Association. 2011;7(3):263-9.

4. Albou P. [Esquirol and dementia]. Hist Sci Med. 2012;46(1):45-53.

5. Boller F, Forbes MM. History of dementia and dementia in history: an overview. Journal of the neurological sciences. 1998;158(2):125-33.

6. Cipriani G, Dolciotti C, Picchi L, Bonuccelli U. Alzheimer and his disease: a brief history. Neurological sciences : official journal of the Italian Neurological Society and of the Italian Society of Clinical Neurophysiology. 2011;32(2):275-9.

7. Dahm R. Alzheimer's discovery. Current biology : CB. 2006;16(21):R906-10.

8. Alzheimer's A. 2016 Alzheimer's disease facts and figures. Alzheimer's & dementia : the journal of the Alzheimer's Association. 2016;12(4):459-509.

9. Herrera E, Jr., Caramelli P, Silveira AS, Nitrini R. Epidemiologic survey of dementia in a community-dwelling Brazilian population. Alzheimer disease and associated disorders. 2002;16(2):103-8.

10. Nitrini R, Caramelli P, Herrera E, Jr., Bahia VS, Caixeta LF, Radanovic M, et al. Incidence of dementia in a community-dwelling Brazilian population. Alzheimer disease and associated disorders. 2004;18(4):241-6.

11. 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.

12. McKhann GM. Changing concepts of Alzheimer disease. JAMA : the journal of the American Medical Association. 2011;305(23):2458-9.

13. 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. Alzheimer's & dementia : the journal of the Alzheimer's Association. 2011;7(3):280-92.

14. 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.

15. Jack CR, Jr., 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 neurology. 2013;12(2):207-16.

16. Petersen RC. Mild cognitive impairment as a diagnostic entity. Journal of internal medicine. 2004;256(3):183-94.

17. Dubois B, Feldman HH, Jacova C, Dekosky ST, Barberger-Gateau P, Cummings J, et al. Research criteria for the diagnosis of Alzheimer's disease: revising the NINCDS-ADRDA criteria. Lancet neurology. 2007;6(8):734-46.

18. Dubois B, Feldman HH, Jacova C, Hampel H, Molinuevo JL, Blennow K, et al. Advancing research diagnostic criteria for Alzheimer's disease: the IWG-2 criteria. Lancet neurology. 2014;13(6):614-29.

19. Schilling L.P, Leuzy A, Zimmer ER, Gauthier S, Rosa-Neto P Nonamyloid PET biomarkers and Alzheimer ' s disease : current and future perspectives. Future Neurology. 2014;9(6):597-613.

20. Nunan J, Small DH. Regulation of APP cleavage by alpha-, beta- and gamma-secretases. FEBS letters. 2000;483(1):6-10.

21. Haass C, Selkoe DJ. Soluble protein oligomers in neurodegeneration: lessons from the Alzheimer's amyloid beta-peptide. Nature reviews Molecular cell biology. 2007;8(2):101-12.

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

23. Williams DR. Tauopathies: classification and clinical update on neurodegenerative diseases associated with microtubule-associated protein tau. Internal medicine journal. 2006;36(10):652-60.

24. Buee L, Bussiere T, Buee-Scherrer V, Delacourte A, Hof PR. Tau protein isoforms, phosphorylation and role in neurodegenerative disorders. Brain research Brain research reviews. 2000;33(1):95-130.

25. Johnson GV, Stoothoff WH. Tau phosphorylation in neuronal cell function and dysfunction. Journal of cell science. 2004;117(Pt 24):5721-9.

26. Gendreau KL, Hall GF. Tangles, Toxicity, and Tau Secretion in AD - New Approaches to a Vexing Problem. Frontiers in neurology. 2013;4:160.

27. Augustinack JC, Schneider A, Mandelkow EM, Hyman BT. Specific tau phosphorylation sites correlate with severity of neuronal cytopathology in Alzheimer's disease. Acta neuropathologica. 2002;103(1):26-35.

28. Ballatore C, Lee VM, Trojanowski JQ. Tau-mediated neurodegeneration in Alzheimer's disease and related disorders. Nature reviews Neuroscience. 2007;8(9):663-72.

29. Spires-Jones TL, Stoothoff WH, de Calignon A, Jones PB, Hyman BT. Tau pathophysiology in neurodegeneration: a tangled issue. Trends in neurosciences. 2009;32(3):150-9.

30. Hardy JA, Higgins GA. Alzheimer's disease: the amyloid cascade hypothesis. Science. 1992;256(5054):184-5.

31. Eikelenboom P, Stam FC. Immunoglobulins and complement factors in senile plaques. An immunoperoxidase study. Acta neuropathologica. 1982;57(2-3):239-42.

32. Eikelenboom P, Hack CE, Rozemuller JM, Stam FC. Complement activation in amyloid plaques in Alzheimer's dementia. Virchows Archiv B, Cell pathology including molecular pathology. 1989;56(4):259-62.

33. Griffin WS, Stanley LC, Ling C, White L, MacLeod V, Perrot LJ, et al. Brain interleukin 1 and S-100 immunoreactivity are elevated in Down syndrome and Alzheimer disease. Proceedings of the National Academy of Sciences of the United States of America. 1989;86(19):7611-5.

34. Akiyama H, Barger S, Barnum S, Bradt B, Bauer J, Cole GM, et al. Inflammation and Alzheimer's disease. Neurobiology of aging. 2000;21(3):383-421.

35. Eikelenboom P, van Gool WA. Neuroinflammatory perspectives on the two faces of Alzheimer's disease. Journal of neural transmission. 2004;111(3):281-94.

36. Serrano-Pozo A, Mielke ML, Gomez-Isla T, Betensky RA, Growdon JH, Frosch MP, et al. Reactive glia not only associates with plaques but also parallels tangles in Alzheimer's disease. The American journal of pathology. 2011;179(3):1373-84.

37. Ferreira ST, Clarke JR, Bomfim TR, De Felice FG. Inflammation, defective insulin signaling, and neuronal dysfunction in Alzheimer's disease. Alzheimer's & dementia : the journal of the Alzheimer's Association. 2014;10(1 Suppl):S76-83.

38. Wyss-Coray T, Mucke L. Inflammation in neurodegenerative disease--a double-edged sword. Neuron. 2002;35(3):419-32.

39. Mrak RE, Griffin WS. Glia and their cytokines in progression of neurodegeneration. Neurobiology of aging. 2005;26(3):349-54.

40. Fuller S, Steele M, Munch G. Activated astroglia during chronic inflammation in Alzheimer's disease--do they neglect their neurosupportive roles? Mutation research. 2010;690(1-2):40-9.

41. de la Monte SM. Quantitation of cerebral atrophy in preclinical and end-stage Alzheimer's disease. Annals of neurology. 1989;25(5):450-9.

42. Rose SE, Chen F, Chalk JB, Zelaya FO, Strugnell WE, Benson M, et al. Loss of connectivity in Alzheimer's disease: an evaluation of white matter tract integrity with colour coded MR diffusion tensor imaging. Journal of neurology, neurosurgery, and psychiatry. 2000;69(4):528-30.

43. Bozzali M, Falini A, Franceschi M, Cercignani M, Zuffi M, Scotti G, et al. White matter damage in Alzheimer's disease assessed in vivo using diffusion tensor magnetic resonance imaging. Journal of neurology, neurosurgery, and psychiatry. 2002;72(6):742-6.

44. Medina D, DeToledo-Morrell L, Urresta F, Gabrieli JD, Moseley M, Fleischman D, et al. White matter changes in mild cognitive impairment and AD: A diffusion tensor imaging study. Neurobiology of aging. 2006;27(5):663-72.

45. Zhang Y, Schuff N, Jahng GH, Bayne W, Mori S, Schad L, et al. Diffusion tensor imaging of cingulum fibers in mild cognitive impairment and Alzheimer disease. Neurology. 2007;68(1):13-9.

46. Nowrangi MA, Lyketsos CG, Leoutsakos JM, Oishi K, Albert M, Mori S, et al. Longitudinal, region-specific course of diffusion tensor imaging measures in mild cognitive impairment and Alzheimer's disease. Alzheimer's & dementia : the journal of the Alzheimer's Association. 2013;9(5):519-28.

47. Reisberg B, Franssen EH, Hasan SM, Monteiro I, Boksay I, Souren LE, et al. Retrogenesis: clinical, physiologic, and pathologic mechanisms in brain aging, Alzheimer's and other dementing processes. European archives of psychiatry and clinical neuroscience. 1999;249 Suppl 3:28-36.

48. Brun A, Gustafson L, Englund E. Subcortical pathology of Alzheimer's disease. Advances in neurology. 1990;51:73-7.

49. Alves GS, Oertel Knochel V, Knochel C, Carvalho AF, Pantel J, Engelhardt E, et al. Integrating retrogenesis theory to Alzheimer's disease pathology: insight from DTI-TBSS investigation of the white matter microstructural integrity. BioMed research international. 2015;2015:291658.

50. Paula-Lima AC, Brito-Moreira J, Ferreira ST. Deregulation of excitatory neurotransmission underlying synapse failure in Alzheimer's disease. Journal of neurochemistry. 2013;126(2):191-202.

51. Revett TJ, Baker GB, Jhamandas J, Kar S. Glutamate system, amyloid ss peptides and tau protein: functional interrelationships and relevance to Alzheimer disease pathology. Journal of psychiatry & neuroscience : JPN. 2013;38(1):6-23.

52. Johnson JW, Kotermanski SE. Mechanism of action of memantine. Current opinion in pharmacology. 2006;6(1):61-7.

53. Peskind ER, Potkin SG, Pomara N, Ott BR, Graham SM, Olin JT, et al. Memantine treatment in mild to moderate Alzheimer disease: a 24-week randomized, controlled trial. The American journal of geriatric psychiatry : official journal of the American Association for Geriatric Psychiatry. 2006;14(8):704-15.

54. van Marum RJ. Update on the use of memantine in Alzheimer's disease. Neuropsychiatric disease and treatment. 2009;5:237-47.

55. Perry EK, Perry RH, Blessed G, Tomlinson BE. Necropsy evidence of central cholinergic deficits in senile dementia. Lancet. 1977;1(8004):189.

56. Francis PT, Palmer AM, Snape M, Wilcock GK. The cholinergic hypothesis of Alzheimer's disease: a review of progress. Journal of neurology, neurosurgery, and psychiatry. 1999;66(2):137-47.

57. Mann DM, Yates PO. Serotonin nerve cells in Alzheimer's disease. Journal of neurology, neurosurgery, and psychiatry. 1983;46(1):96.

58. Palmer AM, Francis PT, Benton JS, Sims NR, Mann DM, Neary D, et al. Presynaptic serotonergic dysfunction in patients with Alzheimer's disease. Journal of neurochemistry. 1987;48(1):8-15.

59. Lanctot KL, Herrmann N, Mazzotta P. Role of serotonin in the behavioral and psychological symptoms of dementia. The Journal of neuropsychiatry and clinical neurosciences. 2001;13(1):5-21.

60. Rosa-Neto P, Hsiung GY, Masellis M, participants C. Fluid biomarkers for diagnosing dementia: rationale and the Canadian Consensus on Diagnosis and Treatment of Dementia recommendations for Canadian physicians. Alzheimer's research & therapy. 2013;5(Suppl 1):S8.

61. Bullmore E, Sporns O. Complex brain networks: graph theoretical analysis of structural and functional systems. Nature reviews Neuroscience. 2009;10(3):186-98.

62. Vitali P, Migliaccio R, Agosta F, Rosen HJ, Geschwind MD. Neuroimaging in dementia. Seminars in neurology. 2008;28(4):467-83.

63. Seab JP, Jagust WJ, Wong ST, Roos MS, Reed BR, Budinger TF. Quantitative NMR measurements of hippocampal atrophy in Alzheimer's disease. Magnetic resonance in medicine. 1988;8(2):200-8.

64. Schilling LP, Zimmer ER, Shin M, Leuzy A, Pascoal TA, Benedet AL, et al. Imaging Alzheimer's disease pathophysiology with PET. Dementia & Neuropsychologia. 2016;10(2):79-90.

65. Klunk WE, Engler H, Nordberg A, Wang Y, Blomqvist G, Holt DP, et al. Imaging brain amyloid in Alzheimer's disease with Pittsburgh Compound-B. Annals of neurology. 2004;55(3):306-19.

66. Scholl M, Damian A, Engler H. Fluorodeoxyglucose PET in Neurology and Psychiatry. PET clinics. 2014;9(4):371-90, v.

67. Pierpaoli C, Basser PJ. Toward a quantitative assessment of diffusion anisotropy. Magnetic resonance in medicine. 1996;36(6):893-906.

68. Le Bihan D, Mangin JF, Poupon C, Clark CA, Pappata S, Molko N, et al. Diffusion tensor imaging: concepts and applications. Journal of magnetic resonance imaging : JMRI. 2001;13(4):534-46.

69. Chua TC, Wen W, Slavin MJ, Sachdev PS. Diffusion tensor imaging in mild cognitive impairment and Alzheimer's disease: a review. Current opinion in neurology. 2008;21(1):83-92.

70. Bartzokis G, Cummings JL, Sultzer D, Henderson VW, Nuechterlein KH, Mintz J. White matter structural integrity in healthy aging adults and patients with Alzheimer disease: a magnetic resonance imaging study. Archives of neurology. 2003;60(3):393-8.

71. Fellgiebel A, Muller MJ, Wille P, Dellani PR, Scheurich A, Schmidt LG, et al. Color-coded diffusion-tensor-imaging of posterior cingulate fiber tracts in mild cognitive impairment. Neurobiology of aging. 2005;26(8):1193-8.

72. Meguro K, Blaizot X, Kondoh Y, Le Mestric C, Baron JC, Chavoix C. Neocortical and hippocampal glucose hypometabolism following neurotoxic lesions of the entorhinal and perirhinal cortices in the non-human primate as shown by PET. Implications for Alzheimer's disease. Brain : a journal of neurology. 1999;122 (Pt 8):1519-31.

73. Bozoki AC, Korolev IO, Davis NC, Hoisington LA, Berger KL. Disruption of limbic white matter pathways in mild cognitive impairment and Alzheimer's disease: a DTI/FDG-PET study. Human brain mapping. 2012;33(8):1792-802.

74. Forster S, Grimmer T, Miederer I, Henriksen G, Yousefi BH, Graner P, et al. Regional expansion of hypometabolism in Alzheimer's disease follows amyloid deposition with temporal delay. Biological psychiatry. 2012;71(9):792-7.

75. Pascoal TA, Mathotaarachchi S, Mohades S, Benedet AL, Chung CO, Shin M, et al. Amyloid-beta and hyperphosphorylated tau synergy drives metabolic decline in preclinical Alzheimer's disease. Molecular psychiatry. 2016.

76. Folstein MF, Folstein SE, McHugh PR. "Mini-mental state". A practical method for grading the cognitive state of patients for the clinician. Journal of psychiatric research. 1975;12(3):189-98.

77. Morris JC. The Clinical Dementia Rating (CDR): current version and scoring rules. Neurology. 1993;43(11):2412-4.

78. Aisen PS, Petersen RC, Donohue MC, Gamst A, Raman R, Thomas RG, et al. Clinical Core of the Alzheimer's Disease Neuroimaging Initiative: progress and plans. Alzheimer's & dementia : the journal of the Alzheimer's Association. 2010;6(3):239-46.

79. Mathotaarachchi S, Wang S, Shin M, Pascoal TA, Benedet AL, Kang MS, et al. VoxelStats: A MATLAB Package for Multi-Modal Voxel-Wise Brain Image Analysis. Frontiers in neuroinformatics. 2016;10:20.

80. Hachinski VC, Iliff LD, Zilhka E, et al. CErebral blood flow in dementia. Archives of neurology. 1975;32(9):632-7.

81. Benjamini; Y, Hochberg Y. Controlling the False Discovery Rate: A Practical and Powerful Approach to Multiple Testing. Journal of the Royal Statistical Society Series B 1995;57(1):289-300.

82. Mielke MM, Kozauer NA, Chan KC, George M, Toroney J, Zerrate M, et al. Regionally-specific diffusion tensor imaging in mild cognitive impairment and Alzheimer's disease. NeuroImage. 2009;46(1):47-55.

83. Agosta F, Pievani M, Sala S, Geroldi C, Galluzzi S, Frisoni GB, et al. White matter damage in Alzheimer disease and its relationship to gray matter atrophy. Radiology. 2011;258(3):853-63.

84. Rowley J, Fonov V, Wu O, Eskildsen SF, Schoemaker D, Wu L, et al. White matter abnormalities and structural hippocampal disconnections in amnestic mild cognitive impairment and Alzheimer's disease. PloS one. 2013;8(9):e74776.

85. Salat DH, Tuch DS, van der Kouwe AJ, Greve DN, Pappu V, Lee SY, et al. White matter pathology isolates the hippocampal formation in Alzheimer's disease. Neurobiology of aging. 2010;31(2):244-56.

86. Reisberg B, Kenowsky S, Franssen EH, Auer SR, Souren LE. Towards a science of Alzheimer's disease management: a model based upon current knowledge of retrogenesis. International psychogeriatrics / IPA. 1999;11(1):7-23.

87. Reisberg B, Franssen EH, Souren LE, Auer SR, Akram I, Kenowsky S. Evidence and mechanisms of retrogenesis in Alzheimer's and other dementias: management and treatment import. American journal of Alzheimer's disease and other dementias. 2002;17(4):202-12.

88. Alves GS, O'Dwyer L, Jurcoane A, Oertel-Knochel V, Knochel C, Prvulovic D, et al. Different patterns of white matter degeneration using multiple diffusion indices and volumetric data in mild cognitive impairment and Alzheimer patients. PloS one. 2012;7(12):e52859.

89. Bosch B, Arenaza-Urquijo EM, Rami L, Sala-Llonch R, Junque C, Sole-Padulles C, et al. Multiple DTI index analysis in normal aging, amnestic MCI and AD. Relationship with neuropsychological performance. Neurobiology of aging. 2012;33(1):61-74.

90. Kantarci K, Schwarz CG, Reid RI, Przybelski SA, Lesnick TG, Zuk SM, et al. White matter integrity determined with diffusion tensor imaging in older adults without dementia: influence of amyloid load and neurodegeneration. JAMA neurology. 2014;71(12):1547-54.

91. Yakushev I, Schreckenberger M, Muller MJ, Schermuly I, Cumming P, Stoeter P, et al. Functional implications of hippocampal degeneration in early Alzheimer's disease: a combined DTI and PET study. European journal of nuclear medicine and molecular imaging. 2011;38(12):2219-27.

92. Villain N, Desgranges B, Viader F, de la Sayette V, Mezenge F, Landeau B, et al. Relationships between hippocampal atrophy, white matter disruption, and gray matter hypometabolism in Alzheimer's disease. The Journal of neuroscience : the official journal of the Society for Neuroscience. 2008;28(24):6174-81.

93. Villain N, Fouquet M, Baron JC, Mezenge F, Landeau B, de La Sayette V, et al. Sequential relationships between grey matter and white matter atrophy and brain metabolic abnormalities in early Alzheimer's disease. Brain : a journal of neurology. 2010;133(11):3301-14.

94. Gold BT, Zhu Z, Brown CA, Andersen AH, LaDu MJ, Tai L, et al. White matter integrity is associated with cerebrospinal fluid markers of Alzheimer's disease in normal adults. Neurobiology of aging. 2014;35(10):2263-71.

95. Stenset V, Bjornerud A, Fjell AM, Walhovd KB, Hofoss D, Due-Tonnessen P, et al. Cingulum fiber diffusivity and CSF T-tau in patients with subjective and mild cognitive impairment. Neurobiology of aging. 2011;32(4):581-9.

96. Li X, Li TQ, Andreasen N, Wiberg MK, Westman E, Wahlund LO. The association between biomarkers in cerebrospinal fluid and structural changes in the brain in patients with Alzheimer's disease. Journal of internal medicine. 2014;275(4):418-27.

97. Zhang Y, Schuff N, Du AT, Rosen HJ, Kramer JH, Gorno-Tempini ML, et al. White matter damage in frontotemporal dementia and Alzheimer's disease measured by diffusion MRI. Brain : a journal of neurology. 2009;132(Pt 9):2579-92.

98. Kantarci K, Avula R, Senjem ML, Samikoglu AR, Zhang B, Weigand SD, et al. Dementia with Lewy bodies and Alzheimer disease: neurodegenerative patterns characterized by DTI. Neurology. 2010;74(22):1814-21.

99. Racine AM, Adluru N, Alexander AL, Christian BT, Okonkwo OC, Oh J, et al. Associations between white matter microstructure and amyloid burden in preclinical Alzheimer's disease: A multimodal imaging investigation. NeuroImage Clinical. 2014;4:604-14.

100. Adluru N, Destiche DJ, Lu SY, Doran ST, Birdsill AC, Melah KE, et al. White matter microstructure in late middle-age: Effects of apolipoprotein E4 and parental family history of Alzheimer's disease. NeuroImage Clinical. 2014;4:730-42.

101. Lowe VJ, Kemp BJ, Jack CR, Jr., Senjem M, Weigand S, Shiung M, et al. Comparison of 18F-FDG and PiB PET in cognitive impairment. Journal of nuclear medicine : official publication, Society of Nuclear Medicine. 2009;50(6):878-86.

102. Jack CR, Jr., Wiste HJ, Weigand SD, Therneau TM, Lowe VJ, Knopman DS, et al. Defining imaging biomarker cut points for brain aging and Alzheimer's disease. Alzheimer's & dementia : the journal of the Alzheimer's Association. 2016.

103. Jack CR, Jr., Albert MS, Knopman DS, McKhann GM, Sperling RA, Carrillo MC, et al. Introduction to the recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. Alzheimer's & dementia : the journal of the Alzheimer's Association. 2011;7(3):257-62.

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. Alzheimer's & dementia : the journal of the Alzheimer's Association. 2011;7(3):270-9.

105. Laforce R, Rosa-Neto P, Soucy JP, Rabinovici GD, Dubois B, Gauthier S. Canadian Consensus Guidelines on Use of Amyloid Imaging in Canada: Update and Future Directions from the Specialized Task Force on Amyloid imaging in Canada. The Canadian journal of neurological sciences Le journal canadien des sciences neurologiques. 2016;43(4):503-12.

106. Hansson O, Zetterberg H, Buchhave P, Londos E, Blennow K, Minthon L. Association between CSF biomarkers and incipient Alzheimer's disease in patients with mild cognitive impairment: a follow-up study. Lancet neurology. 2006;5(3):228-34.

107. Okello A, Koivunen J, Edison P, Archer HA, Turkheimer FE, Nagren K, et al. Conversion of amyloid positive and negative MCI to AD over 3 years: an 11C-PIB PET study. Neurology. 2009;73(10):754-60.

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

109. Scola E, Bozzali M, Agosta F, Magnani G, Franceschi M, Sormani MP, et al. A diffusion tensor MRI study of patients with MCI and AD with a 2-year clinical follow-up. Journal of neurology, neurosurgery, and psychiatry. 2010;81(7):798-805.

110. Fu JL, Liu Y, Li YM, Chang C, Li WB. Use of diffusion tensor imaging for evaluating changes in the microstructural integrity of white matter over 3 years in patients with amnesic-type mild cognitive impairment converting to Alzheimer's disease. Journal of neuroimaging : official journal of the American Society of Neuroimaging. 2014;24(4):343-8.

111. Ito K, Sasaki M, Takahashi J, Uwano I, Yamashita F, Higuchi S, et al. Detection of early changes in the parahippocampal and posterior cingulum bundles during mild cognitive impairment by using high-resolution multi-parametric diffusion tensor imaging. Psychiatry research. 2015;231(3):346-52.

112. Brueggen K, Grothe MJ, Dyrba M, Fellgiebel A, Fischer F, Filippi M, et al. The European DTI Study on Dementia - A multicenter DTI and MRI study on Alzheimer's disease and Mild Cognitive Impairment. NeuroImage. 2016.

113. Andrews-Hanna JR, Smallwood J, Spreng RN. The default network and self-generated thought: component processes, dynamic control, and clinical relevance. Annals of the New York Academy of Sciences. 2014;1316:29-52.

114. Fransson P, Marrelec G. The precuneus/posterior cingulate cortex plays a pivotal role in the default mode network: Evidence from a partial correlation network analysis. NeuroImage. 2008;42(3):1178-84.

115. Leech R, Kamourieh S, Beckmann CF, Sharp DJ. Fractionating the default mode network: distinct contributions of the ventral and dorsal posterior cingulate cortex to cognitive control. The Journal of neuroscience : the official journal of the Society for Neuroscience. 2011;31(9):3217-24.

116. Kochunov P, Ramage AE, Lancaster JL, Robin DA, Narayana S, Coyle T, et al. Loss of cerebral white matter structural integrity tracks the gray matter metabolic decline in normal aging. NeuroImage. 2009;45(1):17-28.

117. Kadir A, Almkvist O, Forsberg A, Wall A, Engler H, Langstrom B, et al. Dynamic changes in PET amyloid and FDG imaging at different stages of Alzheimer's disease. Neurobiology of aging. 2012;33(1):198 e1-14.

ANEXO A - Artigo 1: Alzheimer's and Parkinson's diseases: An environmental proteomic point of view

JOURNAL OF PROTEOMICS 104 (2014) 24–36

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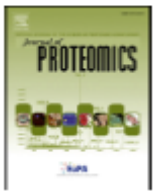

Review

Alzheimer's and Parkinson's diseases: An environmental proteomic point of view☆

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ARTICLE INFO

Available online 18 April 2014

Keywords:
Alzheimer's
Parkinson's
Biomarkers
Environmental factors
Neurodegenerative diseases
Mass spectrometry

ABSTRACT

Alzheimer's and Parkinson's diseases are severe neurodegenerative conditions triggered by complex biochemical routes. Many groups are currently pursuing the search for valuable biomarkers to either perform early diagnostic or to follow the disease's progress. Several studies have reported relevant findings regarding environmental issues and the progression of such diseases. Here the etiology and mechanisms of these diseases are briefly reviewed. Approaches that might reveal candidate biomarkers and environmental stressors associated to the diseases were analyzed under a proteomic perspective.
This article is part of a Special Issue entitled: Environmental and structural proteomics.

Contents

☆ This article is part of a Special Issue entitled: Environmental and structural proteomics.

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http://dx.doi.org/10.1016/j.jprot.2014.04.014
1874-3919/

1. Introduction

Proteomic approaches are widely used in biology, medicine, agriculture and many other areas. The main idea, regardless of the biological question behind, is to understand the expression, quantification, compartmentalization, mobilization, or modification of proteins under a specific condition. The types and numbers of these conditions vary extensively: development, biotic and abiotic stress, diseases, medical treatment, and so on. (See Figs. 1 and 2.)

Reports on environmental studies using proteomic approaches have increased in the past years. In these cases, "satellite" organisms aided to monitor different kinds of stresses caused by environmental conditions, such as water, air or soil pollution, intoxication by different poisons, heavy metals, organic solvents, ionizing radiation, and electromagnetic field [1–3].

Although there is a wide range of neurodegenerative diseases (NDs), in this review, the neurodegenerative disorders Parkinson's (PD) and Alzheimer's diseases (AD) were chosen to illustrate proteomic approaches and studies focusing on environmental proteomics.

2. Environmental proteomics

Environmental changes caused by different stressors can be studied applying proteomic approaches. These strategies can reflect the physiological response of living beings to changing conditions or stressful environmental states [4]. Minimal alterations on the environment may lead to important adaptations of organisms to this new condition. As pointed out by González-Fernández and collaborators, environmental proteomics encompasses studies on toxic and defense mechanisms triggered by different pollutants, without previous knowledge about the biological systems themselves, which is one of the advantages of this approach [5]. Although proteomic studies can compare dynamic responses in several conditions, only in recent years has this strategy gained space in environmental issues, particularly biomarker searches for intoxication/contamination, or environmental risk factors [6].

Examples of studies performed in which the "environmental problem" was addressed using proteomics, include terrestrial ecosystems [2], semimetal intoxication [7], and exposure to tobacco smoke [8]. In a work performed by Montes-Nieto and collaborators using *Mus pretus* as a bioindicator, the protein expression profile of animals from Domingo Rubio stream was compared to that of animals from Doñana Biological Reserve (both in Spain), using 2-DE (two-dimensional electrophoresis) and peptide mass fingerprinting by MALDI-TOF (matrix-assisted laser-desorption ionization-time-of-flight). Relevant differences in the animal's proteome were identified, including proteins with a defensive role against the toxic and polluted environment as well as proteins that could make them more susceptible [9].

In a more recent publication also employing 2-DE as protein fractionation method, Company and co-workers compared subproteomes of the mussel *Bathymodiolus azoricus*. This animal lives in a gradient zone at the bottom of the oceans, in which water from the hydrothermal vents mixes with sea water, characterized by extreme variable conditions of pH, high metals and salt contents, and wide oscillations in temperature. Besides these extreme conditions, several reducing chemical species are present in this environment, which can cause severe oxidative damages through generation of reactive oxygen species (ROS). The authors selected mussels from different locations, and performed an enrichment of thiol-containing proteins, by using an activated thiol Sepharose matrix. Proteomic analysis was performed by 2-DE only, without protein identification by mass spectrometry. The authors found a correlation between thiol direct oxidation by ROS and the site of collection [10].

Dieldrin, a powerful organochloride pesticide which blocks gamma-amino-butyric acid (GABA) receptors in the CNS, was widely used in the 1960–1980s. This pesticide is very lipophilic, and accumulates in fish fat and muscle. In a study by Martyniuk and colleagues, gene expression analysis by microarray and iTRAQ were combined to quantitatively evaluate proteins differentially expressed in largemouth bass fishes fed on subchronic dieldrin-containing diets. The applied proteomic approach revealed decrease in the levels of seven proteins and increase of eleven other proteins in the dieldrin-fed group. Several of the identified proteins are known to be involved in human NDs, such as microtubule-associated tau protein, myelin basic protein, enolase 1, stathmin 1a, apolipoprotein E, and parvalbumin. Martyniuk's study has shown that dieldrin affected "pathological pathways" shared by both AD and PD, overlapping with proteomic signatures known for these neurological diseases [11], which are related to energy production, protection from oxidative damage, and synapse integrity. The authors suggested that "common pathways could be activated by stress or injury of the CNS and may be the result of apoptosis, inflammation, and oxidative damage that may precede neurotoxicity and neural damage" [12].

The effects of another important toxic agent, arsenic, was evaluated using SELDI-TOF (surface-enhanced laser desorption/ionization). This semimetal has high affinity to sulfhydryl groups in keratin, and can be detected in high amounts in the skin, hair and nails of intoxicated individuals [7]. In the study by Harezlak and coauthors, plasma samples from a population in Bangladesh known to be exposed to As were analyzed and an extensive questionnaire was applied to the subjects in order to understand their lifestyle. Authors used a

"unified statistical method that simultaneously takes into account different sources of variation that are present in mass spectrometry measurements". The raw data was decomposed into four different stages: baseline, signal, instrumental noise, and random noise. The authors concluded that the 20 superproteins (protein peaks which fitted into the criteria) detected in the population could be used as an early diagnostic for As exposure, and that the statistical method proposed could be expanded to LC-MS and MALDI-TOF approaches [7].

Considering toxicants related to central nervous system, a proteomic approach identified differential protein expression in the cortex of rats after cocaine exposure [13]. In this case, Guan and Guan studied the medial prefrontal cortex, which is highly activated after cocaine exposure, which can lead to irreversible changes in this brain's area. The authors used a conditioned place preference assay in rats, as a model for addictive drugs. Protein was extracted and analyzed by 2-DE and MALDI-TOF/TOF. There were about 71 differentially expressed spots between control and "addicted" groups, belonging to different functional classes and perhaps these proteins could serve as models or targets to understand cocaine addiction [13].

3. Neurodegenerative diseases and proteomics

Neurodegenerative diseases (NDs) are incurable conditions that result in progressive degeneration or loss of neurons in the affected individuals [14]. The occurrence of NDs is

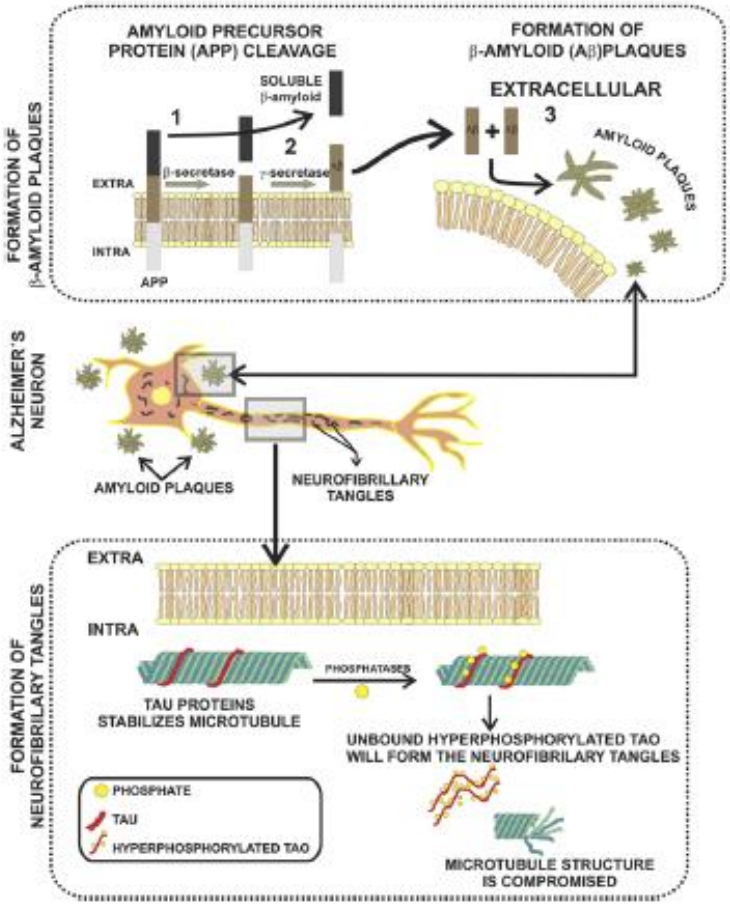

Fig. 1 – In the figure, the formation of β-amyloid plaques is presented in the upper panel. The amyloid precursor protein (APP) is cleaved by β- and γ-secretase and produces one soluble and another insoluble fraction (Aβ). The Aβ aggregation will produce the amyloid plaques. Lower panel: tau protein (TAU) is found in the microtubules and helps the stabilization of this structure. However, hyperphosphorylated TAU causes destabilization of the microtubule because unbound hyperphosphorylated tau aggregates and as result, the microfibrillary tangles are produced. Both (amyloid and tangles) will cause severe damage to the neuron cell.

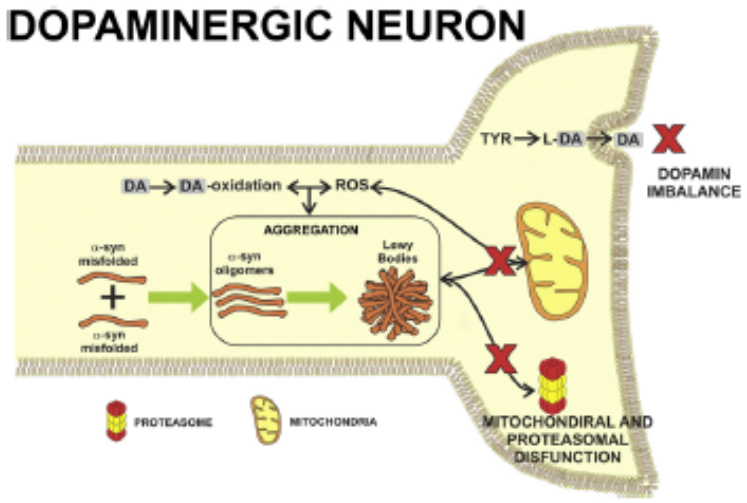

Fig. 2– In Alzheimer's disease, the pathological process starts at the brain region known as *substantia nigra pars compacta*. It is accepted that alpha-synuclein (α-syn) aggregation will form the Lewy bodies. The α-syn aggregation process will produce severe mitochondrial and proteasomal dysfunction. Reactive oxygen species (ROS) produced in and imbalanced way will contribute to the overall process and at the end. The regular dopamine (DA) metabolism, from tyrosine, levo dopa (L-DOPA) and finally DA will be imbalanced as a result of the entire process and the Parkinson disease will be established. The figure presents a simple representation of a much more complex disease.

significantly increasing over the past decades, especially because of the global increase in life expectancy. Nowadays there is a great interest in understanding the pathogenesis of these diseases, aiming to detect very early signs and symptoms, discover preclinical biomarkers and to develop new therapies for stopping and/or reverting the underlying processes. Some of these diseases constitute a major challenge for health professionals, as very often diagnosis is given only in advanced stages and, despite the different physiopathological processes underlying the diseases, usually these conditions share several clinical symptoms [15–17]. The development of effective diagnostic methods, which could identify patients at risk and the early stages of these illnesses would be of major importance. The early diagnosis of ND diseases ideally should include central nervous system imaging and biomarkers from different sources, such as blood and cerebrospinal fluid (CSF), that could support the clinical diagnosis [18]. A biomarker is defined as a 'characteristic that is objectively measured and evaluated as an indicator of normal biology, pathological process, or pharmacologic responses to a therapeutic intervention' [19]. According to Pal and colleagues, presently no biomarkers exist for reliable diagnosis, tracking of disease progression or monitoring responses to treatment regimes [20]. Therefore, the search for valuable biomarkers for diagnostics and prognostics of NDs are of great interest worldwide [21].

Proteomic approaches have been extensively applied to discover new biomarkers for early diagnostics and prognostics for these diseases. The best "source" to obtain a reliable biomarker for neurodegenerative diseases is the CSF. However, as pointed by Shi and colleagues, the biomarker might be present in the CFS only at later stages of NDs, no longer being useful for early diagnosis or intervention. Hence it is desirable that a biomarker would first be detectable in the blood and subsequently in the CSF, if possible [22].

Proteomic approaches applied in studies of the CNS performed with embryonic and postnatal brain tissue, different brain regions, CSF, neural stem cells, pre and post synaptic proteomes and neurodegenerative diseases were extensively reviewed by Zang [23]. One very interesting and elegant investigation was carried out by Bernay and colleagues, aimed to study the secretome of the CNS; in different words, the contents of the extracellular compartment. A secretome of rat striatum was obtained by performing microdialysis to collect proteins and peptides that participate in the complex network of communication within and between brain regions [24]. In this study, the microdialysis fluid (secretome) was fractionated into proteins of >100 kDa, >10 kDa and <100 kDa and peptides around 20 kDa. Two different mass spectrometers, an Orbitrap (Thermo) and a Q-TOF Ultima (Waters) were used for the analysis. According to the authors, the differential pre-fractionation methods and combination of two mass spectrometers were essential for the success of the study, in which they detected for the rat striatum secretome about 88 proteins and 100 peptides (derived from 29 different protein precursors), potentially involved in signaling of the complex brain network [24].

More than 300 proteins were found altered in brain and CFS of ND patients or other psychiatric condition (the studies comprised Alzheimer's disease, Parkinson's disease, Down's syndrome, Pick's disease, Creutzfeldt–Jakob disease, schizophrenia, bipolar disorder, depression, hypoxia, ischemia and neuropathic pain). This compilation, reviewed in details by Fountoulakis, comprised mainly qualitative studies performed

either by 2-DE followed by MALDI-TOF or LC–MS/MS analyses [25].

Neurodegenerative diseases were also studied under redox proteomic approaches. A comprehensive review by Butterfield about the topic leads to similar results: proteins involved in glucose metabolism, mitochondrial function, structural, and protein degradation are commonly affected in some NDs (Alzheimer's disease, Parkinson's disease, Huntington's disease and amyotrophic lateral sclerosis), suggesting that there might be a shared mechanism by which neurodegeneration takes place in different diseases [26].

4. Environmental agents and NDs

Many metals can be found in the environment in different forms. Some severe conditions can develop caused by the excessive ingestion or absorption of metals, including acute toxicity, mental retardation, antibiotic resistance and even death. The etiology of several diseases might be related to previous exposure to heavy metals or other intoxicant agents [27–30].

The central nervous system is very sensitive to different agents, and among them, copper can be cited. In the CNS, there is a low level of important antioxidant enzymes, contrasting with a high level of easily oxidized substrates and combined with a high flux of ROS that are generated during neurochemical reactions [31]. Despite its importance in biochemistry, Cu^{+2} ions may disrupt the correct conformation of some peptides and proteins. It is known that Cu^{+2} induces conformational change in the normal prion protein, which modify from a random coil into β-sheet characteristic of the PrP form of the protein, which is associated with Prion diseases [32,33]. Cu^{+2} ions can contribute to the formation of the β-sheet or extended conformation of amyloidal beta peptides, which are associated with AD [33]. On the other hand, α-helical structures are important for the formation of paired helical filaments. It is assumed that in AD, Cu^{+2} participates of the formation of this motif in neurofibril tangles. The stoichiometry of copper binding to peptide R2, one of the four highly conserved regions of tau protein (R1 to R4), was studied by MALDI-TOF and the formation of the R2–Cu^{+2} complex was confirmed although being less strong than the R3–Cu^{+2} complex [32]. Copper is found in several types of wires widely used worldwide and workers exposed to them are susceptible to chronic intoxication.

In another case-control study, Gorell and coworkers analyzed the potential role of occupational exposure to iron, copper, manganese, mercury, zinc, and lead as risk factors for PD, and found a significantly increased association of the disease in patients with more than 20 years of exposure with copper and manganese. The author also reported a greater association of PD with exposure to combinations of lead–copper, iron–copper and lead–iron than with any of these metals alone [34].

High levels of some metals in the brain, including aluminum, zinc and iron levels may also be linked to the development or progression of AD [35]. Zatta and colleagues reviewed the role of these metals in neurodegenerative processes. While aluminum is reported as a very controversial cofactor in AD and other NDs, manganese apparently plays an important role in causing PD, with increased environmental burden of manganese being associated with neurodegeneration in the basal ganglia. The author also reported no evidences associating zinc to ND [36].

Recently reviewed by Bakusky and colleagues, lead exposure can also be associated with AD. Lead can be absorbed by the lung epithelium and gastrointestinal tract, upon binding to heme groups and consequently can flow around the body through the blood [37]. In short words, early episodes in life and/or continuous exposure to lead can contribute to amyloidogenesis in later life stages [37,38]. In the study performed by Basha and co-workers using rodents, the authors observed that lead exposure induced transient suppression of the β-amyloid precursor protein in neonates, followed by a delayed over expression 20 months after the exposure ceased [38].

The role of environmental factors in the incidence of NDs has been addressed by analyzing mainly pesticide exposure, in special in relation to Parkinson and Alzheimer's disease [39,40]. People who live in a rural area, drinks well water, and works in activities related to farming are more exposed to pesticides from different sources, which may be a risk factor for developing PD [40].

In a prospective cohort in which 1507 French elderly were followed, Baldi and colleagues identified an increased relative risk to develop NDs in subjects who had been occupationally exposed to different pesticides (insecticides, herbicides, and fungicides). However, it was not possible to correlate one specific chemical to the development of any neurological disorder. The focus was given to AD and PD and the "diagnostic" was given based on a simple algorithm based on a standardized questionnaire that classified the subjects as either suspected or not suspected of having dementia [41].

Back in 1997, in a case–control study in Taiwan, Liou and coworkers reported that PD risk was greater among subjects exposed to paraquat and other herbicides/pesticides than those not exposed. However, the author did not find significant differences in occupational exposures to chemicals, heavy metals, and minerals among PD patients and matched control subjects [42].

In case of AD, the contribution of environmental factors is controversial. Some authors report an increased risk to develop AD associated to occupational exposure to pesticides [43,44]. On the other hand, different authors failed to show such risk [45]. The controversy of these studies might be due to the fact that susceptibility to pesticides and other neurotoxins depends on variability in xenobiotic metabolism, possibly generated by genetic polymorphisms, aging and degree of exposure to environmental agents [46].

There are other atypical Parkinson's-like syndromes beyond PD. Multiple System Atrophy (MSA) and Progressive Supranuclear Palsy (PSP) are neurodegenerative conditions with clinical features similar to PD, which may be confused considering their clinical aspects. While MSA is a disease associated to α-synuclein accumulation [47] PSP seems to be a tau pathology [15]. MSA incidence has been associated with metal dusts and fumes, plastic monomers and additives, organic solvents, and pesticides [48]. Dexter and colleagues reported an increased concentration of iron patients with MSA and also PSP, suggesting a possible environmental factor in these diseases [49].

Oxidative stress is inherent of several regular physiological processes, in which ROS are generated. Several environmental pollutants, including heavy metals and pesticides potentially exacerbate ROS production [50]. The imbalance on ROS production and physiological antioxidant mechanism, caused by external agents can contribute to the etiology or progress of several diseases, such as AD. Copper, chromium and cadmium are known to cause protein damage through ROS intermediates, and were studied by redox proteomic approaches [50]. Nitrated proteins, specifically enolase, glyceraldehyde-3-phosphate dehydrogenase, ATP synthase, carbonic anhydrase-II and voltage-dependent anion channel were detected by Sultana and colleagues, in frozen hippocampal samples from AD patients analyzed by 2-DE followed by MALDI-TOF [51].

5. Alzheimer's disease

The most common form of age-related neurodegenerative disease in the world is Alzheimer's disease (AD), the leading cause of dementia [52]. In 2006, the worldwide prevalence of AD was 26.6 million and estimates are that by 2050, prevalence will quadruple [53], bringing a very high socioeconomic impact and requiring huge adjustments of governments, social agencies, health insurances and families to deal with these patients.

In 1906 the German neurologist Alois Alzheimer (1864–1915) first described the clinical and pathological features of an unusual brain disease during the Tübingen Assembly of Southwest German Psychiatry [54,55]. His presentation described the case of Auguste Deter, who, at age 51, presented with a rapidly progressive dementia syndrome. Post-mortem examination revealed the presence of amyloid plaques and neurofibrillary tangles. These findings were published by Alzheimer in 1907, in the form of a short report. In 1910, the psychiatrist Emil Kraepelin, a colleague of Alzheimer, introduced the term Alzheimer's disease (AD) in his Handbook of Psychiatry.

The two core pathological hallmarks of AD are amyloid plaques and neurofibrillary tangles. The amyloid cascade hypothesis suggests that the deposition of amyloid β (Aβ) peptide triggers neuronal dysfunction and cell death in the brain. Tau, a microtubule-associated protein, is the major constituent of neurofibrillary tangles. The amyloid cascade hypothesis proposes that changes in tau and consequent neurofibrillary tangle formation are triggered by toxic concentrations of Aβ [56]. Due to the difficulty in the early diagnosis of this disease, in recent years much effort has been made in the discovery of biomarkers for AD, which could allow the disease to be diagnosed at an early stage [57].

Previously, the AD diagnosis included exclusively patients on the dementia stage [58], and the disease was characterized by histology pathological features. Currently, AD is conceptualized as a progressive pathophysiological process in which the accumulation of β-amyloid (Aβ) pathology is thought to set in motion a dynamic sequential cascade of events, including neurodegeneration, inflammatory processes, and neurotransmitter dysfunction. Even the clinical aspects have changed in the last clinical diagnosis consensus, including also preclinical stages and incorporating biomarkers to support the diagnosis [59].

With the current conceptual changes that redefined AD as process, the physiopathological onset, duration and the underlying mechanisms of AD have received great attention of specialized researchers. It is hypothesized that the pathologic process of AD begins around two decades before cognitive decline [60], and may vary among individuals. The most common primary symptom of AD is a decline in cognitive functions, known as mild cognitive impairment (MCI), with deficits minimally interfering in activities of daily life [56,61]. At the MCI stage, considered as an AD prodromal phase [62], biomarkers have a crucial role, in revealing the onset of the pathophysiological process and urging clinical interventions, which are today still on the clinical trial phase. All identified potential biomarkers are still in the testing stage and clinical studies based on large population studies are needed [63].

Diverse approaches searching for AD biomarkers were reported, including plasma proteomics, plasma lipidomics, transcriptome, autoantibodies, microRNA, plasma Aβ species, and plasma tau differential forms [64]. Studies of plasma lipidomics derived from findings that the deregulation of lipid pathways could be implicated in AD [65] and transcriptome profiling-based methods have been used in an attempt to identify a blood-based signature (a serum biomarker) to differentiate AD patients from asymptomatic control subjects [66–68]. Although the presence of autoantibodies in AD has been demonstrated, their role in the pathology of disease is still unclear [69]. There is substantial evidence that alterations in microRNA levels are associated with some parts of AD pathology, however its relevance as a blood-based biomarker requires validation [70]. Some post translational modifications have been identified as potential biochemical markers to measure the disease's activity. Increased levels of oxidative modification markers have been demonstrated; recently, mitochondria isolated from lymphocytes of MCI patients were shown to present signs of increased oxidative stress, which may potentially reflect brain damage and serve as a biomarker for AD [71].

Cerebrospinal fluid and positron emission tomography (PET) are the current clinical biomarkers used to confirm AD pathologic changes in patients diagnosed as having dementia. The use of CSF biomarkers is widely discussed. Rosa-Neto et al. recommended considering it at a tertiary care level to improve diagnostic certainty, particularly in those cases presenting atypical clinical features [72].

CSF biomarkers such as amyloid-β 1-42 (Aβ42), total tau (t-tau) and phosphorylated tau (p-tau) are "hallmarks" of the disease, reflecting axonal damage, and phosphorylated tau (p-tau) indicating neurofibrillary tangle pathology [73–75]. CSF tau is considered to be a strong marker of the neuronal injury associated with AD, and the combined detection of Aβ42, t-tau and p-tau levels in CSF are considered to have a high diagnostic accuracy even in the early stages of Alzheimer's disease [76]. Wang and colleagues reported that "decreased cerebrospinal fluid Aβ42 and increased CSF phosphorylated tau_{181} were independently associated with reduced default mode network integrity with the most prominent decreases in functional connectivity observed between the posterior cingulate and medial temporal regions" [77]. The combination of

low CSF Aβ42 and elevated tau in CSF also correlates with higher risks of progression to AD in patients with MCI [78]. CSF biomarkers are thus thought to be useful in the very early diagnosis of AD [75].

Albeit extremely useful, the CSF collection procedure is very invasive, as it requires a lumbar puncture and adequate infrastructure to perform this procedure. Because blood sampling, in contrast to CSF, is less invasive and thus more accepted by patients, biomarkers in blood are highly desirable and helpful in monitoring follow up [14]. Plasma biomarkers combined with baseline demographics have been suggested as a potential screening tool [79]. Urine and saliva have also been tested as possible analytes in AD research.

5.1. Proteomics in Alzheimer's disease

Presently most of the AD proteomic data report findings on proteins derived from CSF or blood, either using protein arrays or mass spectrometry-based detection of blood profiles [80–82]. Several promising blood-based biomarkers of AD have been proposed in studies ranging from proteomic analysis in plasma to genetic profiling [64], among which are apolipoprotein E (with controversies), brain natriuretic peptide, pancreatic polypeptide and C-reactive protein [83].

Doecke and coworkers identified a panel of plasma biomarkers that distinguish individuals with AD from cognitively healthy control subjects with high sensitivity and specificity. These include biomarkers with significantly increased levels (cortisol, pancreatic polypeptide, insulin-like growth factor binding protein 2, β2 microglobulin, vascular cell adhesion molecule 1, carcinoembryonic antigen, matrix metalloprotein 2, CD40, macrophage inflammatory protein 1α, superoxide dismutase, and homocysteine) and biomarkers with significantly decreased levels (apolipoprotein E, epidermal growth factor receptor, hemoglobin, calcium, zinc, interleukin 17, and albumin) in AD [84]. Others studies based on plasma proteome evaluated apolipoprotein A-II, apolipoprotein E (ApoE), serum glutamic oxaloacetic transaminase, α-1-microglobulin and brain natriuretic peptide for AD diagnosis [85]. Apolipoprotein E, immunoglobulin M, eotaxin-3, N-terminal prohormone of brain natriuretic peptide, matrix metalloproteinase 1, pancreatic polypeptide and tenascin-C were evaluated by Soares and colleagues in MCI and AD patients. Their results confirmed studies reporting CSF increased levels of pancreatic polypeptide, eotaxin 3, tenascin C and NT-pro BNP in patients with AD and MCI [79]. Ray and colleagues reported that several serum signaling proteins, as chemokine (C–C motif) ligand-5, -7, -15, and -18; chemokine (C–X–C motif) ligand-8; epidermal growth factor; granulocyte colony stimulating factor; glial-derived neurotrophic factor; intracellular adhesion molecule 1; insulin-like growth factor binding protein 6; interleukin 1α, 3 and 11; macrophage colony-stimulating factor; platelet derived growth factor-BB; tumor necrosis factor α and tumor necrosis related apoptosis-inducing ligand R4 are associated to AD and progression of MCI to AD [86].

As mentioned previously, ApoE is a candidate for an AD biomarker even though some discrepancies have been found when the levels of this protein are related to AD symptoms. Aiming to clarify this point, Simon and colleagues applied the powerful mass spectrometry quantitative approach of selected reaction monitoring (SRM) to quantify ApoE and ApoE4 in AD patients and control subjects. By using 'proteotypic' peptides (cysteine 112–cysteine 158 in ApoE; cysteine 112–arginine 158 in ApoE4) characteristic of each isoform, which differ in only one amino acid, the authors concluded from this target mass spectrometry approach that ApoE and ApoE4 are not clinically significant relevant for AD diagnostics [87].

Adding to the controversy on ApoE and ApoE4 as markers for AD, Wang and colleagues analyzed the same isoforms under a multiple reaction monitoring approach (MRM). In this case, the authors performed protein quantification in the soluble and insoluble cell fractions. Their data have shown that C and N-terminal fragments of ApoE and ApoE4 accumulate in higher amounts in AD tissues, but the full assignment of these fragments identities has not yet be done. The approach used in Wag's study provided "quantitative evidence for a preferable accumulation of apoE C-terminal fragment in the insoluble fraction of AD frontal cortex homogenate" [88].

Zhang and colleagues performed SRM to study histone acetylation in human brain tissue of advanced AD patients, using samples from individuals at different stages of the disease [89]. A considerable lower amount of histone acetylation in AD samples was detected when compared to controls, pointing to the need of further studies to understand the participation of this post-translational protein modification in AD evolution.

Domenico and colleagues performed a quantitative proteomic study, by measuring the levels of phosphorylated proteins in hippocampus of AD patients. Hyperphosphorylation of tau proteins is considered to be a hallmark of AD [90]. According to the authors, several other proteins could also be erroneously phosphorylated and contribute to the evolution of AD [90]. In this work a critical point has been identified, that the PMI period (post modem interval) could influence the outcome of proteomic studies. During the time interval or delay between "death" and "sample" collection, several processes, i.e. proteolysis, unrelated to any pathological process might happen. Although control samples are used for comparison, the delay still inevitably exists. To avoid this situation, the search for biomarkers in alive individuals should continue. The authors reported 17 proteins differentially phosphorylated in AD samples as compared to control samples; nine of them presented increased phosphorylation in AD subjects [90]. In this study, samples were pre-fractionated by 2-DE, and analyzed in an Orbitrap. No phosphopeptide enrichment was performed. Protein phosphorylation was estimated based on ProQ dym staining. The altered phosphorylation patterns in AD patients occurred in proteins involved in energy metabolism and ATP production, signal transduction and neural structure, all key steps for AD development and evolution [90].

The nitration of proteins in tyrosine residues potentially interferes with phosphorylation processes which are vital for many biological functions. Nitrated proteins detected in Sultana's study suggested imbalance of energy metabolism, synaptic loss, and mitochondrial dysfunction, all phenomena nowadays accepted as part of mechanisms leading to AD. Di Domenico and colleagues investigated the role of cellular stress response on AD progression, by evaluating the levels

of HSP 27, 32, 60, 70 and 90 and thioredoxin-1 by Western blot [91]. The main idea of this study was to measure important proteins related to stress response and protein folding. The samples were autopsied from subjects belonging to the aMCI (amnestic) stage at a maximum 3 h PMI, and three brain regions were analyzed: hippocampus, inferior parietal lobule and cerebellum. In general, the HSP levels were higher in AD samples when compared to control samples, except in the cerebellum. HSP 32 was detected at the highest amount, but without significant difference between the groups [91].

Another interesting approach was used by Reed and co-workers in the analysis of proteins that could bind to 4-hydroxy-2-nonenal (HNE) in the human brain. HNE has an important role in lipid peroxidation and can cause oxidative stress in the brain in its free form or bound to proteins [80]. The study compared subjects at two stages of the disease, the mild cognitive impairment (MCI) phase and late-stage Alzheimer's disease. The analysis of HNE-bound proteins was performed by immunohistochemistry and selected proteins were analyzed by MALDI-TOF, after preparation by 2-DE. The authors pointed out that lipid peroxidation seems to have an important role in AD, even in the early stages MCI and EAD (early Alzheimer's disease) [80]. More recently, Hashimoto and colleagues analyzed microdissected hippocampal neurons by O^{18} labeling mass spectrometry. The elegant approach of laser capture microdissection (LCM) allowed the authors to analyze specific neurons population of the brain tissue. LCM was used to extract post mortem neurons of the *cornu ammonis* 1 region from AD and control patients. The *cornu ammonis* 1 is where neurofibrilar tangles are detected even in early stages of AD. The approach allowed detection of up- and down-regulated neuron-specific proteins, when comparing AD to control samples. Through this strategy, one very specific region of the brain could be analyzed in a way that precluded proteins from regions, not implicated in the pathology, to interfere in the proteomics study [92].

6. Parkinson's disease

The English physician James Parkinson in his article "An Essay on the Shaking Palsy" first described the clinical features of this condition in 1817. Parkinson's disease (PD) is the second major neurodegenerative disease in the world, which affects about 0.3% of the general population including all ethnic and socioeconomic groups, with a slight predominance in males. Its incidence and prevalence increase with age, reaching about 1% in people above 60 years and 4% in those above 80 years [93]. Clinically, PD manifests as resting tremor, bradykinesia (difficulty in initiating movements and slow to run them) and muscle rigidity. These changes usually have an asymmetrical onset. Difficulties in gait and postural instability and autonomic dysfunction are symptoms that may be associated with disease progression [17].

The depletion of dopamine in PD is one of the hallmarks of the disease. The postmortem brain pathological evaluation shows the degeneration of *substantia nigra* in the *pars compacta*, leading to dopamine deficiency. Cytoplasmic eosinophilic inclusions termed Lewy bodies (LB), composed mainly of α-synuclein are also found in areas of neuronal degeneration in these patients [94].

Although a definitive diagnosis is only given in the autopsy, the syndromic diagnosis is based on clinical criteria, being the UK Parkinson's Disease Society Brain Bank the most used globally [16]. The PD is not limited to motor disorders as cognitive deficits can also be detected. Studies indicate a 30% prevalence of dementia in individuals with PD, and it is estimated that at least 75% of patients with more than ten years of disease progression develop dementia [95]. PD patients who develop dementia during the first year have been classified as having dementia with Lewy bodies (DLB) [96]. The risk of developing dementia in PD is particularly high in patients older than 70 years [97,98].

In the past years there is an increasing consensus in that exposure to toxicants such as heavy metals, pesticides and other known neurotoxic substances can increase the risk of developing PD [99]. The idea that neurodegeneration, such as that observed in PD, is closely related to oxidative stress is now accepted [99]. Symptoms similar to those of PD patients have been detected in subjects exposed to manganese [99–101]. The accumulation of aluminum in the brain of PD patients was described, and increased incidence of neurological diseases, including PD, correlates to high levels of aluminum in drinking water [102]. Several techniques are available to quantify metals in different tissues. The laser ablation inductively coupled plasma mass spectrometry is one of these strategies. In a work performed by Matush and colleagues, they analyzed MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine)-exposed mice using this technique to analyze details of Cu, Fe, Zn and Mn mobilization in brain tissues [103].

Exposures to pesticides have been associated with PD in several aspects. The associations of PD with rotenone and paraquat, two worldwide-used pesticides known to easily cross the blood brain barrier, have been reported [104]. The effect of some pesticides, including, paraquat, rotenone and dieldrin over the α-synuclein were studied by Uversky and colleagues (in vitro). The authors used atomic force microscopy to study the α-synuclein conformation and fibril formation, respectively, after treatment with the pesticides. As result, a significant conformational change in the α-synudein structure and formation of α-synuclein fibrils in a high rate were observed, suggesting strong participation of these pesticides in the development of Parkinson disease [105].

Rotenone inhibits the complex I of the electron transport chain in mitochondria thus disturbing the oxidative phosphorylation process [30,50]. Rotenone intoxication promotes selective degeneration of *nigral* dopaminergic neurons with accumulation of cytoplasmic α-synuclein aggregates, resulting in symptoms seen in PD, such as bradykinesia, rigidity, tremor and nonmotor signs [30,104].

The herbicide paraquat has been pointed as a stronger environmental factor in PD occurrence [106]. Paraquat generates ROS through production of superoxide radicals and induces a parkinsonian syndrome similar in many features to PD. It also increases lipid peroxidation, decreases levels of antioxidants, disturbs mitochondrial function, increases expression and aggregation of α-synuclein and selectively kills nigral dopaminergic neurons [30,107,108].

Under the histopathological point of view, the tau protein, the amyloid protein and Lewy bodies are involved in the development of PD dementia [95,109,110]. The identification of biomarkers that will allow an earlier and more accurate diagnosis in PD with dementia or DLB is urgent as the population ages globally increased [111]. Chang and coworkers suggested that microRNA biomarkers associated with AD could have potentials for other neurodegenerative diseases as well, such as in Parkinson's, Prion and Huntington's diseases [112]. Other studies have proposed that combining clinical findings, biochemical and imaging markers (MRI, PET and SPECT) will be more likely to contribute to early PD diagnosis and follow up [113,114].

6.1. Proteomics in Parkinson's disease

Biomarkers for diagnosis and prognosis of PD are not currently available. Actually, some putative candidates for PD biomarkers were proposed but these still have low specificity and sensitivity [18]. Promising findings in the field showed that α-synuclein is a major component of Lewis bodies [115]; that DJ-1 is involved in protection against oxidative stress during ND [116] and that levels of Aβ42 correlate with cognitive impairment.

The current "omic" approaches in PD include transcriptomics, proteomics and metabolomics, aimed at identifying small changes in mRNA, protein or metabolite profiles [114]. Searches for biomarkers in PD were performed in CSF (α-synuclein, tau, β-amyloid peptides and DJ-1) and proteins and urate in the blood [18,113,117]. Recently it has been suggested that tau and β amyloid are critically involved in early PD progression, probably by a mechanism different than that in Alzheimer's disease [118].

The rat ventral mesencephalic tissue gives rise to the dopamine neurons within the *substantia nigra*, which degenerates in Parkinson's disease. Using a quantitative proteomic approach (iTRAQ), Orme and collaborators studied protein expression in tissues in three different stages: immediately before, during and after the dopaminergic neurogenesis. Briefly, extracted total protein was labeled with iTRAQ reagents, submitted to trypsin digestion followed by peptide pre-fractionation using multidimensional chromatography (strong cation exchange + reverse phase). Using this strategy, the authors identified ca. 3000 proteins by MALDI-TOF/TOF and could explore in details the proteins involved in the dopamine neuron development [119].

Proteomics approaches have been used to understand the role of mitochondria perturbation and oxidative stress in the role of the PD [120]. Van Laar and collaborators analyzed by 2-DIGE (fluorescence difference gel electrophoresis) rat brain mitochondria after exposure to dopamine–quinone (DAQ). In a healthy brain, dopamine (DA) leads to the production of ROS and DA which is not adequately stored in vesicles can be oxidized to form the reactive DAQ [121]. Increased levels of cysteinyl-DA, a covalent modification of DA triggered by DAQ, have been detected post-mortem in *substantia nigra* of PD patients. Furthermore it has been demonstrated that DAQ can cause alteration of respiratory mechanisms in mitochondria [121]. Proteins oxidized in the mitochondria, according to the authors, could also be potential targets for therapeutic agents in AD. The authors performed mitochondrial isolation from brain tissue of rats and the extracted mitochondrial proteins were analyzed by 2-DIGE coupled to MALDI TOF/TOF mass spectrometry. Mitochondrial creatine kinase (MtCK), which is associated with ADP–ATP exchange and the permeability transition pore [122], is highly sensitive to oxidation. Their data showed that DA-induced oxidation of MtCK affected its enzyme activity thus, compromising the integrity and energy metabolism of mitochondria [121].

Parkinsonism mouse models can be obtained by the use of two neurotoxins: 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) and methamphetamine (METH). MPTP seems to cause death of dopaminergic neurons in the *substantia nigra pars compacta* [123], while METH inhibits oxidative phosphorylation in dopaminergic neurons [124,125]. Analysis of these two systems under trancriptomic and proteomic approaches revealed significant changes on the levels of 86 proteins and of mRNA of 181 genes after toxin(s) treatment(s). The authors concluded that there is a clear mitochondrial dysfunction in PD, with increased oxidative stress, deregulated protein degradation, increased apoptosis and cell death, and a potential activation of the astrocytic response. This study was performed with $^{16}O/^{18}O$ labeling and Cys-peptide fractionation, aiming an accurate quantification and a wide coverage of the proteome [125].

Constantinescu and colleagues analyzed the CSF from patients with different clinical stages of parkinsonian disorders. The authors compared the protein profile of these groups aiming to identify one or more specifics biomarkers by mass spectrometry SELDI-TOF (using three different surface arrays: cation and anion exchange and metal binding). However, no specific biomarker could be detected and used to distinguish the pathologies among the groups. The authors pointed out that SELDI might not be a good strategy, because the detection limit of the approach could be limiting the detection of low abundant proteins [126].

Several studies have pointed to α-synuclein as a biomarker candidate for PD. Even though this small protein can be detected in the plasma, there are still many controversies regarding its use as a biomarker [127]. Chen and colleagues compared the plasma of healthy and PD patient groups, using 2-DE and a Q-TOF mass spectrometer. Abundant proteins were depleted from blood samples and analyzed it separately. A significant difference between the groups for the proteins serum amyloid component P and IgGκL prompted the authors to suggest these two proteins as biomarker candidates. Although ELISA further confirmed these results, the group of PD patients analyzed in the study was small hence more studies are necessary to validate the data [127].

As a matter of fact, a recent systematic review of biomarkers concluded that there is still insufficient evidence to recommend the use of any biomarker for disease progression in PD clinical trials [128].

7. Concluding remarks

This review has briefly highlighted some physiopathological aspects of Parkinson's and Alzheimer's diseases, the most common age-related neurodegenerative diseases in the world. The occurrence of these diseases has significantly increased

over the past decades, in parallel to global increase in life expectancy. Presently no biomarkers exist for reliable diagnosis, tracking of disease progression or monitoring therapeutic outcomes. Here emphasis was given in reviewing proteomic studies aiming at identification of the urgently needed biomarker(s) that will allow an early detection and subsequent therapeutic intervention to deter the progress or at least ameliorate the symptoms of these debilitating pathologies. The role of environmental stressors in the incidence of these neurodegenerative diseases was addressed and proteomic studies dealing with understanding the effects and diagnosing the exposure to different pollutants and heavy metals in relation to Parkinson and Alzheimer's disease were reviewed. The controversial data on candidate biomarkers useful for diagnostic and prognostic of these CNS diseases reveal a field with many gaps yet to be solved, in which proteomic approaches have ensured application.

Acknowledgments

The authors would like to express their gratitude to the editors of the special issue for the invitation and acceptance of the proposed subject. The authors are grateful to the Brazilian funding agencies *Conselho Nacional de Desenvolvimento Científico e Tecnológico* (CNPq, Ministry of Science and Technology-Universal 2012, # 47.5908/2012-0), *Coordenação de Aperfeiçoamento de Pessoal de Nível Superior* (CAPES, Ministry of Education- Toxinologia number 63/2010, # 1205/2011), and *Fundação de Amparo à Pesquisa do Estado do Rio Grande do Sul* (FAPERGS, PRONEX, # 10/0014-2).

REFERENCES

[1] Vineis P, van Veldhoven K, Chadeau-Hyam M, Athersuch TJ. Advancing the application of omics-based biomarkers in environmental epidemiology. Environ Mol Mutagen 2013;54:461–7.
[2] Dowd WW. Challenges for biological interpretation of environmental proteomics data in non-model organisms. Integr Comp Biol 2012;52:705–20.
[3] Brocchieri L. Environmental signatures in proteome properties. Proc Natl Acad Sci U S A 2004;101:8257–8.
[4] Gotelli NJ, Ellison AM, Ballif BA. Environmental proteomics, biodiversity statistics and food-web structure. Trends Ecol Evol 2012;27:436–42.
[5] González-Fernández M, García-Barrera T, Arias-Borrego A, Jurado J, Pueyo C, López-Barea J, et al. Metallomics integrated with proteomics in deciphering metal-related environmental issues. Biochimie 2009;91:1311–7.
[6] Pedriali A, Riva C, Parolini M, Cristoni S, Sheehan D, Binelli A. A redox proteomic investigation of oxidative stress caused by benzoylecgonine in the freshwater bivalve *Dreissena polymorpha*. Drug Test Anal 2013;5:646–56.
[7] Harezlak J, Wu MC, Wang M, Schwartzman A, Christiani DC, Lin X. Biomarker discovery for arsenic exposure using functional data. Analysis and feature learning of mass spectrometry proteomic data. J Proteome Res 2008;7:217–24.
[8] Fuller BF, Gold MS, Wang KKW, Ottens AK. Effects of environmental tobacco smoke on adult rat brain biochemistry. J Mol Neurosci 2010;41:165–71.
[9] Montes-Nieto R, Fuentes-Almagro CA, Bonilla-Valverde D, Prieto-Alamo MJ, Jurado J, Carrascal M, et al. Proteomics in free-living *Mus spretus* to monitor terrestrial ecosystems. Proteomics 2007;7:4376–87.
[10] Company R, Torreblanca A, Cajaraville M, Bebianno MJ, Sheehan D. Comparison of thiol subproteome of the vent mussel *Bathymodiolus azoricus* from different Mid-Atlantic Ridge vent sites. Sci Total Environ 2012;437:413–21.
[11] De Iuliis A, Grigoletto J, Recchia A, Giusti P, Arslan P. A proteomic approach in the study of an animal model of Parkinson's disease. Clin Chim Acta 2005;357:202–9.
[12] Martyniuk CJ, Kroll KJ, Doperalski NJ, Barber DS, Denslow ND. Genomic and proteomic responses to environmentally relevant exposures to dieldrin: indicators of neurodegeneration? Toxicol Sci 2010;117:190–9.
[13] Guan X, Guan Y. Proteomic profile of differentially expressed proteins in the medial prefrontal cortex after repeated cocaine exposure. Neuroscience 2013;236:262–70.
[14] Genius J, Klafki H, Benninghoff J, Esselmann H, Wiltfang J. Current application of neurochemical biomarkers in the prediction and differential diagnosis of Alzheimer's disease and other neurodegenerative dementias. Eur Arch Psychiatry Clin Neurosci 2012;262(Suppl. 2):S71–7.
[15] Respondek G, Roeber S, Kretzschmar H, Troakes C, Al-Sarraj S, Gelpi E, et al. Accuracy of the National Institute for Neurological Disorders and Stroke/Society for Progressive Supranuclear Palsy and neuroprotection and natural history in Parkinson plus syndromes criteria for the diagnosis of progressive supranuclear palsy. Mov Disord 2013;28:504–9.
[16] Hughes AJ, Daniel SE, Ben-Shlomo Y, Lees AJ. The accuracy of diagnosis of parkinsonian syndromes in a specialist movement disorder service. Brain 2002;125:861–70.
[17] Gelb DJ, Oliver E, Gilman S. Diagnostic criteria for Parkinson disease. Arch Neurol 1999;56:33–9.
[18] Parnetti L, Castrioto A, Chiasserini D, Persichetti E, Tambasco N, El-Agnaf O, et al. Cerebrospinal fluid biomarkers in Parkinson disease. Nat Rev Neurol 2013;9:131–40.
[19] Atkinson AJ, Colburn WA, DeGruttola VG, DeMets DL, Downing GJ, Hoth DF, et al. Biomarkers and surrogate endpoints: preferred definitions and conceptual framework. Clin Pharmacol Ther 2001;69:89–95.
[20] Pal R, Alves G, Larsen JP, Moller SG. New insight into neurodegeneration: the role of proteomics. Mol Neurobiol 2013:1–19. http://dx.doi.org/10.1007/s12035-013-8590-8.
[21] Tsuruoka M, Hara J, Hirayama A, Sugimoto M, Soga T, Shankle WR, et al. Capillary electrophoresis-mass spectrometry-based metabolome analysis of serum and saliva from neurodegenerative dementia patients. Electrophoresis 2013;34(19):2865–72.
[22] Shi M, Caudle WM, Zhang J. Biomarker discovery in neurodegenerative diseases: a proteomic approach. Neurobiol Dis 2009;35:157–64.
[23] Zhang C. Proteomic studies on the development of the central nervous system and beyond. Neurochem Res 2010;35:1487–500.
[24] Bernay B, Gaillard MC, Guryča V, Emadali A, Kuhn L, Bertrand A, et al. Discovering new bioactive neuropeptides in the striatum secretome using in vivo microdialysis and versatile proteomic. Mol Cell Proteomics 2009;8:946–58.
[25] Fountoulakis M, Kossida S. Proteomics-driven progress in neurodegeneration research. Electrophoresis 2006;27:1556–73.
[26] Butterfield DA, Perluigi M, Reed T, Muharib T, Hughes CP, Robinson RAS, et al. Redox proteomics in selected neurodegenerative disorders: from its infancy to future applications. Antioxid Redox Signal 2012;17:1610–55.

[27] Benninghoff AD. Toxicoproteomics — the next step in the evolution of environmental biomarkers? Toxicol Sci 2007;95:1–4.
[28] Bjørnstad A, Larsen BK, Skadsheim A, Jones MB, Andersen OK. The potential of ecotoxicoproteomics in environmental monitoring: biomarker profiling in mussel plasma using ProteinChip array technology. J Toxicol Environ Health A 2006;69:77–96.
[29] Dowling VA, Sheehan D. Proteomics as a route to identification of toxicity targets in environmental toxicology. Proteomics 2006;6:5597–604.
[30] Goldman SM. Environmental toxins and Parkinson's disease. Annu Rev Pharmacol Toxicol 2014;54:141–64.
[31] Ozcelik D, Uzun H. Copper intoxication; antioxidant defenses and oxidative damage in rat brain. Biol Trace Elem Res 2009;127:45–52.
[32] Ma Q, Li Y, Du J, Liu H, Kanazawa K, Nemoto T, et al. Copper binding properties of a tau peptide associated with Alzheimer's disease studied by CD, NMR, and MALDI-TOF MS. Peptides 2006;27:841–9.
[33] Jones CE, Abdelraheim SR, Brown DR, Viles JH. Preferential Cu^{2+} coordination by His96 and His111 induces beta-sheet formation in the unstructured amyloidogenic region of the prion protein. J Biol Chem 2004;279:32018–27.
[34] Gorell JM, Johnson CC, Rybicki BA, Peterson EL, Kortsha GX, Brown GG, et al. Occupational exposure to manganese, copper, lead, iron, mercury and zinc and the risk of Parkinson's disease. Neurotoxicology 1999;20:239–47.
[35] Shcherbatykh I, Carpenter DO. The role of metals in the etiology of Alzheimer's disease. J Alzheimers Dis 2007;11:191–205.
[36] Zatta P, Lucchini R, van Rensburg SJ, Taylor A. The role of metals in neurodegenerative processes: aluminum, manganese, and zinc. Brain Res Bull 2003;62:15–28.
[37] Bakulski KM, Rozek LS, Dolinoy DC, Paulson HL, Hu H. Alzheimer's disease and environmental exposure to lead: the epidemiologic evidence and potential role of epigenetics. Curr Alzheimer Res 2012;9:563–73.
[38] Basha MR, Wei W, Bakheet SA, Benitez N, Siddiqi HK, Ge YW, et al. The fetal basis of amyloidogenesis: exposure to lead and latent overexpression of amyloid precursor protein and beta-amyloid in the aging brain. J Neurosci 2005;25:823–9.
[39] Grant WB, Campbell A, Itzhaki RF, Savory J. The significance of environmental factors in the etiology of Alzheimer's disease. J Alzheimers Dis 2002;4:179–89.
[40] Priyadarshi A, Khuder SA, Schaub EA, Priyadarshi SS. Environmental risk factors and Parkinson's disease: a metaanalysis. Environ Res 2001;86:122–7.
[41] Baldi I, Lebailly P, Mohammed-Brahim B, Letenneur L, Dartigues JF, Brochard P. Neurodegenerative diseases and exposure to pesticides in the elderly. Am J Epidemiol 2003;157:409–14.
[42] Liou HH, Tsai MC, Chen CJ, Jeng JS, Chang YC, Chen SY, et al. Environmental risk factors and Parkinson's disease: a case–control study in Taiwan. Neurology 1997;48:1583–8.
[43] Hayden KM, Norton MC, Darcey D, Ostbye T, Zandi PP, Breitner JC, et al. Occupational exposure to pesticides increases the risk of incident AD: the Cache County study. Neurology 2010;74:1524–30.
[44] Tyas SL, Manfreda J, Strain LA, Montgomery PR. Risk factors for Alzheimer's disease: a population-based, longitudinal study in Manitoba, Canada. Int J Epidemiol 2001;30:590–7.
[45] Gauthier E, Fortier I, Courchesne F, Pepin P, Mortimer J, Gauvreau D. Environmental pesticide exposure as a risk factor for Alzheimer's disease: a case–control study. Environ Res 2001;86:37–45.
[46] Le Couteur DG, McLean AJ, Taylor MC, Woodham BL, Board PG. Pesticides and Parkinson's disease. Biomed Pharmacother 1999;53:122–30.
[47] Gilman S, Wenning GK, Low PA, Brooks DJ, Mathias CJ, Trojanowski JQ, et al. Second consensus statement on the diagnosis of multiple system atrophy. Neurology 2008;71:670–6.
[48] Nee LE, Gomez MR, Dambrosia J, Bale S, Eldridge R, Polinsky RJ. Environmental-occupational risk factors and familial associations in multiple system atrophy: a preliminary investigation. Clin Auton Res 1991;1:9–13.
[49] Dexter DT, Carayon A, Javoy-Agid F, Agid Y, Wells FR, Daniel SE, et al. Alterations in the levels of iron, ferritin and other trace metals in Parkinson's disease and other neurodegenerative diseases affecting the basal ganglia. Brain 1991;114(Pt 4):1953–75.
[50] Braconi D, Bernardini G, Santucci A. Linking protein oxidation to environmental pollutants: redox proteomic approaches. J Proteomics 2011;74:2324–37.
[51] Sultana R, Poon HF, Cai J, Pierce WM, Merchant M, Klein JB, et al. Identification of nitrated proteins in Alzheimer's disease brain using a redox proteomics approach. Neurobiol Dis 2006;22:76–87.
[52] Thies W, Bleiler L, Alzheimer A. Alzheimer's disease facts and figures. Alzheimers Dement 2013;9:208–45.
[53] Brookmeyer R, Johnson E, Ziegler-Graham K, Arrighi HM. Forecasting the global burden of Alzheimer's disease. Alzheimers Dement 2007;3:186–91.
[54] Cipriani G, Dolciotti C, Picchi L, Bonuccelli U. Alzheimer and his disease: a brief history. Neurol Sci 2011;32:275–9.
[55] Dahm R. Alzheimer's discovery. Curr Biol 2006;16:R906–10.
[56] Ballard C, Gauthier S, Corbett A, Brayne C, Aarsland D, Jones E. Alzheimer's disease. Lancet 2011;377:1019–31.
[57] Borroni B, Di Luca M, Padovani A. Predicting Alzheimer dementia in mild cognitive impairment patients. Are biomarkers useful? Eur J Pharmacol 2006;545:73–80.
[58] 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;34:939–44.
[59] McKhann GM, Knopman DS, Chertkow H, Hyman BT, Jack Jr 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;7:263–9.
[60] 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;12:357–67.
[61] Gauthier S, Reisberg B, Zaudig M, Petersen RC, Ritchie K, Broich K, et al. International Psychogeriatric Association Expert Conference on mild cognitive I. Mild cognitive impairment. Lancet 2006;367:1262–70.
[62] Dubois B, Feldman HH, Jacova C, Dekosky ST, Barberger-Gateau P, Cummings J, et al. Research criteria for the diagnosis of Alzheimer's disease: revising the NINCDS-ADRDA criteria. Lancet Neurol 2007;6:734–46.
[63] Song F, Poljak A, Smythe GA, Sachdev P. Plasma biomarkers for mild cognitive impairment and Alzheimer's disease. Brain Res Rev 2009;61:69–80.
[64] Henriksen K, O'Bryant SE, Hampel H, Trojanowski JQ, Montine TJ, Jeromin A, et al. The future of blood-based biomarkers for Alzheimer's disease. Alzheimers Dement 2014;10:115–31.
[65] Di Paolo G, Kim TW. Linking lipids to Alzheimer's disease: cholesterol and beyond. Nat Rev Neurosci 2011;12:284–96.

[66] Booij BB, Lindahl T, Wetterberg P, Skaane NV, Saebo S, Feten G, et al. A gene expression pattern in blood for the early detection of Alzheimer's disease. J Alzheimers Dis 2011;23:109–19.

[67] Fehlbaum-Beurdeley P, Sol O, Desire L, Touchon J, Dantoine T, Vercelletto M, et al. Validation of AclarusDx, a blood-based transcriptomic signature for the diagnosis of Alzheimer's disease. J Alzheimers Dis 2012;32:169–81.

[68] Rye PD, Booij BB, Grave G, Lindahl T, Kristiansen L, Andersen HM, et al. A novel blood test for the early detection of Alzheimer's disease. J Alzheimers Dis 2011;23:121–9.

[69] Colasanti T, Barbati C, Rosano G, Malorni W, Ortona E. Autoantibodies in patients with Alzheimer's disease: pathogenetic role and potential use as biomarkers of disease progression. Autoimmun Rev 2010;9:807–11.

[70] Geekiyanage H, Jicha GA, Nelson PT, Chan C. Blood serum miRNA: non-invasive biomarkers for Alzheimer's disease. Exp Neurol 2012;235:491–6.

[71] Sultana R, Baglioni M, Cecchetti R, Cai J, Klein JB, Bastiani P, et al. Lymphocyte mitochondria: toward identification of peripheral biomarkers in the progression of Alzheimer disease. Free Radic Biol Med 2013;65:595–606.

[72] Rosa-Neto P, Hsiung GY, Masellis M, Participants C. Fluid biomarkers for diagnosing dementia: rationale and the Canadian Consensus on Diagnosis and Treatment of Dementia recommendations for Canadian physicians. Alzheimers Res Ther 2013;5:58.

[73] Dubois B, Feldman HH, Jacova C, Cummings JL, Dekosky ST, Barberger-Gateau P, et al. Revising the definition of Alzheimer's disease: a new lexicon. Lancet Neurol 2010;9:1118–27.

[74] Henry MS, Passmore AP, Todd S, McGuinness B, Craig D, Johnston JA. The development of effective biomarkers for Alzheimer's disease: a review. Int J Geriatr Psychiatry 2013;28:331–40.

[75] Kang JH, Korecka M, Toledo JB, Trojanowski JQ, Shaw LM. Clinical utility and analytical challenges in measurement of cerebrospinal fluid amyloid-beta(1-42) and tau proteins as Alzheimer disease biomarkers. Clin Chem 2013;59:903–16.

[76] Blennow K, Zetterberg H. The application of cerebrospinal fluid biomarkers in early diagnosis of Alzheimer disease. Med Clin North Am 2013;97:369–76.

[77] Wang L, Brier MR, Snyder AZ, Thomas JB, Fagan AM, Xiong C, et al. Cerebrospinal fluid Abeta42, phosphorylated Tau181, and resting-state functional connectivity. JAMA Neurol 2013;70:1242–8.

[78] 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;7:270–9.

[79] Soares HD, Potter WZ, Pickering E, Kuhn M, Immermann FW, Shera DM, et al. Plasma biomarkers associated with the apolipoprotein E genotype and Alzheimer disease. Arch Neurol 2012;69:1310–7.

[80] Reed TT, Pierce WM, Markesbery WR, Butterfield DA. Proteomic identification of HNE-bound proteins in early Alzheimer disease: insights into the role of lipid peroxidation in the progression of AD. Brain Res 2009;1274:66–76.

[81] Zurbig P, Jahn H. Use of proteomic methods in the analysis of human body fluids in Alzheimer research. Electrophoresis 2012;33:3617–30.

[82] Lista S, Faltraco F, Hampel H. Biological and methodical challenges of blood-based proteomics in the field of neurological research. Prog Neurobiol 2013;101–102:18–34.

[83] Hu WT, Holtzman DM, Fagan AM, Shaw LM, Perrin R, Arnold SE, et al. Alzheimer's disease neuroimaging I. Plasma multianalyte profiling in mild cognitive impairment and Alzheimer disease. Neurology 2012;79:897–905.

[84] Doecke JD, Laws SM, Faux NG, Wilson W, Burnham SC, Lam CP, et al. Blood-based protein biomarkers for diagnosis of Alzheimer disease. Arch Neurol 2012;69:1318–25.

[85] Llano DA, Devanarayan V, Simon AJ, Alzheimer's Disease Neuroimaging I. Evaluation of plasma proteomic data for Alzheimer disease state classification and for the prediction of progression from mild cognitive impairment to Alzheimer disease. Alzheimer Dis Assoc Disord 2013;27:233–43.

[86] Ray S, Britschgi M, Herbert C, Takeda-Uchimura Y, Boxer A, Blennow K, et al. Classification and prediction of clinical Alzheimer's diagnosis based on plasma signaling proteins. Nat Med 2007;13:1359–62.

[87] Simon R, Girod M, Fonbonne C, Salvador A, Clément Y, Lantéri P, et al. Total ApoE and ApoE4 isoform assays in an Alzheimer's disease case-control study by targeted mass spectrometry (n = 669): a pilot assay for methionine-containing proteotypic peptides. Mol Cell Proteomics 2012;11:1389–403.

[88] Wang M, Turko IV. Mass spectrometry quantification revealed accumulation of C-terminal fragment of apolipoprotein E in the Alzheimer's frontal cortex. PLoS One 2013;8.

[89] Zhang K, Schrag M, Crofton A, Trivedi R, Vinters H, Kirsch W. Targeted proteomics for quantification of histone acetylation in Alzheimer's disease. Proteomics 2012;12:1261–8.

[90] Di Domenico F, Sultana R, Barone E, Perluigi M, Cini C, Mancuso C, et al. Quantitative proteomics analysis of phosphorylated proteins in the hippocampus of Alzheimer's disease subjects. J Proteomics 2011;74:1091–103.

[91] Di Domenico F, Sultana R, Tiu GF, Scheff NN, Perluigi M, Cini C, et al. Protein levels of heat shock proteins 27, 32, 60, 70, 90 and thioredoxin-1 in amnestic mild cognitive impairment: an investigation on the role of cellular stress response in the progression of Alzheimer disease. Brain Res 2010;1333:72–81.

[92] Hashimoto M, Bogdanovic N, Nakagawa H, Volkmann I, Aoki M, Winblad B, et al. Analysis of microdissected neurons by ^{18}O mass spectrometry reveals altered protein expression in Alzheimer's disease. J Cell Mol Med 2012;16:1686–700.

[93] de Lau LM, Breteler MM. Epidemiology of Parkinson's disease. Lancet Neurol 2006;5:525–35.

[94] Hornykiewicz O. The discovery of dopamine deficiency in the parkinsonian brain. J Neural Transm Suppl 2006:9–15.

[95] Aarsland D, Kurz MW. The epidemiology of dementia associated with Parkinson's disease. Brain Pathol 2010;20:633–9.

[96] McKeith IG, Dickson DW, Lowe J, Emre M, O'Brien JT, Feldman H, et al. Diagnosis and management of dementia with Lewy bodies: third report of the DLB Consortium. Neurology 2005;65:1863–72.

[97] Williams-Gray CH, Foltynie T, Brayne CE, Robbins TW, Barker RA. Evolution of cognitive dysfunction in an incident Parkinson's disease cohort. Brain 2007;130:1787–98.

[98] Buter TC, van den Hout A, Matthews FE, Larsen JP, Brayne C, Aarsland D. Dementia and survival in Parkinson disease: a 12-year population study. Neurology 2008;70:1017–22.

[99] Bocca B, Alimonti A, Petrucci F, Violante N, Sancesario G, Forte G, et al. Quantification of trace elements by sector field inductively coupled plasma mass spectrometry in urine, serum, blood and cerebrospinal fluid of patients with Parkinson's disease. Spectrochim Acta Part B At Spectrosc 2004;59:559–66.

[100] Witholt R, Gwiazda RH, Smith DR. The neurobehavioral effects of subchronic manganese exposure in the presence and absence of pre-parkinsonism. Neurotoxicol Teratol 2000;22:851–61.

[101] Verity MA. Manganese neurotoxicity: a mechanistic hypothesis. Neurotoxicology 1999;20:489–97.
[102] Flaten TP. Geographical associations between aluminium in drinking water and death rates with dementia (including Alzheimer's disease), Parkinson's disease and amyotrophic lateral sclerosis in Norway. Environ Geochem Health 1990;12:152–67.
[103] Matusch A, Depboylu C, Palm C, Wu B, Höglinger GU, Schäfer MKH, et al. Cerebral bioimaging of Cu, Fe, Zn, and Mn in the MPTP mouse model of Parkinson's disease using laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS). J Am Soc Mass Spectrom 2010;21:161–71.
[104] Cannon JR, Tapias V, Na HM, Honick AS, Drolet RE, Greenamyre JT. A highly reproducible rotenone model of Parkinson's disease. Neurobiol Dis 2009;34:279–90.
[105] Uversky VN, Li J, Fink AL. Pesticides directly accelerate the rate of alpha-synuclein fibril formation: a possible factor in Parkinson's disease. FEBS Lett 2001;500:105–8.
[106] Dinis-Oliveira RJ, Remiao F, Carmo H, Duarte JA, Navarro AS, Bastos ML, et al. Paraquat exposure as an etiological factor of Parkinson's disease. Neurotoxicology 2006;27:1110–22.
[107] McCormack AL, Thiruchelvam M, Manning-Bog AB, Thiffault C, Langston JW, Cory-Slechta DA, et al. Environmental risk factors and Parkinson's disease: selective degeneration of nigral dopaminergic neurons caused by the herbicide paraquat. Neurobiol Dis 2002;10:119–27.
[108] Kuter K, Nowak P, Golembiowska K, Ossowska K. Increased reactive oxygen species production in the brain after repeated low-dose pesticide paraquat exposure in rats. A comparison with peripheral tissues. Neurochem Res 2010;35:1121–30.
[109] Kempster PA, O'Sullivan SS, Holton JL, Revesz T, Lees AJ. Relationships between age and late progression of Parkinson's disease: a clinico-pathological study. Brain 2010;133:1755–62.
[110] Horvath J, Herrmann FR, Burkhard PR, Bouras C, Kovari E. Neuropathology of dementia in a large cohort of patients with Parkinson's disease. Parkinsonism Relat Disord 2013;19:864–8 [discussion 64].
[111] Johansen KK, White LR, Sando SB, Aasly JO. Biomarkers: Parkinson disease with dementia and dementia with Lewy bodies. Parkinsonism Relat Disord 2010;16:307–15.
[112] Cheng L, Quek CY, Sun X, Bellingham SA, Hill AF. The detection of microRNA associated with Alzheimer's disease in biological fluids using next-generation sequencing technologies. Front Genet 2013;4:150.
[113] Sharma S, Moon CS, Khogali A, Haidous A, Chabenne A, Ojo C, et al. Biomarkers in Parkinson's disease (recent update). Neurochem Int 2013;63:201–29.
[114] Schapira AH. Recent developments in biomarkers in Parkinson disease. Curr Opin Neurol 2013;26:395–400.
[115] Irvine GB, El-Agnaf OM, Shankar GM, Walsh DM. Protein aggregation in the brain: the molecular basis for Alzheimer's and Parkinson's diseases. Mol Med 2008;14:451–64.
[116] Waragai M, Wei J, Fujita M, Nakai M, Ho GJ, Masliah E, et al. Increased level of DJ-1 in the cerebrospinal fluids of sporadic Parkinson's disease. Biochem Biophys Res Commun 2006;345:967–72.
[117] da Costa AG, Gago MF, Garrett C. Biomarcadores no líquido cefalorraquidiano para o diagnóstico precoce de doença de Parkinson. Acta Med Port 2011;24:761–8.
[118] Zhang J, Mattison HA, Liu C, Ginghina C, Auinger P, McDermott MP, et al. Longitudinal assessment of tau and amyloid beta in cerebrospinal fluid of Parkinson disease. Acta Neuropathol 2013;126:671–82.
[119] Orme RP, Gates MA, Fricker-Gates RA. A multiplexed quantitative proteomics approach for investigating protein expression in the developing central nervous system. J Neurosci Methods 2010;191:75–82.
[120] Jin J, Meredith GE, Chen L, Zhou Y, Xu J, Shie FS, et al. Quantitative proteomic analysis of mitochondrial proteins: relevance to Lewy body formation and Parkinson's disease. Mol Brain Res 2005;134:119–38.
[121] Van Laar VS, Dukes AA, Cascio M, Hastings TG. Proteomic analysis of rat brain mitochondria following exposure to dopamine quinone: implications for Parkinson disease. Neurobiol Dis 2008;29:477–89.
[122] Beutner G, Ruck A, Riede B, Brdiczka D. Complexes between porin, hexokinase, mitochondrial creatine kinase and adenylate translocator display properties of the permeability transition pore. Implication for regulation of permeability transition by the kinases. Biochim Biophys Acta 1998;1368:7–18.
[123] Chin MH, Qian WJ, Wang H, Petyuk VA, Bloom JS, Sforza DM, et al. Mitochondrial dysfunction, oxidative stress, and apoptosis revealed by proteomic and transcriptomic analyses of the striata in two mouse models of Parkinson's disease. J Proteome Res 2008;7:666–77.
[124] Lotharius J, Brundin P. Pathogenesis of Parkinson's disease: dopamine, vesicles and alpha-synuclein. Nat Rev Neurosci 2002;3:932–42.
[125] Chin MH, Qian WJ, Wang H, Petyuk VA, Bloom JS, Sforza DM, et al. Mitochondrial dysfunction, oxidative stress, and apoptosis revealed by proteomic and transcriptomic analyses of the striata in two mouse models of Parkinson's disease. J Proteome Res 2008;7:666–77.
[126] Constantinescu R, Andreasson U, Li S, Podust VN, Mattsson N, Anckarsäter R, et al. Proteomic profiling of cerebrospinal fluid in parkinsonian disorders. Parkinsonism Relat Disord 2010;16:545–9.
[127] Chen HM, Lin CY, Wang V. Amyloid P component as a plasma marker for Parkinson's disease identified by a proteomic approach. Clin Biochem 2011;44:377–85.
[128] McGhee DJ, Royle PL, Thompson PA, Wright DE, Zajicek JP, Counsell CE. A systematic review of biomarkers for disease progression in Parkinson's disease. BMC Neurol 2013;13:35.

Discussão Artigo 1

O artigo intitulado “Alzheimer’s and Parkinson’s diseases: An environmental proteomic point of view” é um trabalho de revisão publicado no Journal of Proteomics em 2014, que teve como objetivo revisar os mecanismos e marcadores relacionados com a DA e a Doença de Parkinson sob uma perspectiva focada especialmente nos processos relacionados com proteínas envolvidas nessas doenças.

Inicialmente o artigo revisa aspectos da proteômica, área que estuda os processos associados a alterações nas proteínas, como expressão, quantificação, compartimentalização e mobilização, sob uma condição específica. Com a finalidade de ilustrar as diferentes abordagens e perspectivas geradas através de estudos com enfoque proteômico, foram escolhidas duas das principais doenças neurodegenerativas e revisadas possíveis associações com aspectos ambientais como fatores de risco para o desenvolvimento de tais condições. Através de uma análise de potenciais fatores ambientais, como radiação, poluição e substâncias tóxicas foram revisadas alterações que possam levar ao desequilíbrio metabólico associados com essas doenças. Sob o ponto de vista proteômico na análise de aspectos da DA, diversos estudos descrevem processos inflamatórios como fatores associados ao desequilíbrio de diferentes sistemas metabólicos protéicos na doença.

Neste primeiro estudo realizado dentro do período do doutorado, o enfoque foi voltado aos biomarcadores obtidos através da análise de fluidos, como por exemplo a análise de níveis das proteínas amiloide β_{1-42} e tau no líquido cefalorraquidiano, método já incorporado na abordagem clínico-científica da DA. A perspectiva gerada através de estudos na área da proteômica contribui para o desenvolvimento de possíveis biomarcadores plasmáticos que não demandem procedimentos invasivos, como uma punção lombar, para obtenção de uma alíquota para análise. Através dessa estratégia, diversos aspectos relacionados com a DA poderiam ser avaliados através da análise de um painel de diferentes proteínas e metabólitos relacionados com a doença, cujas informações serviriam para avaliação de risco, diagnóstico precoce e monitorização de abordagens terapêuticas.

ANEXO B - Artigo 2: Nonamyloid PET biomarkers and Alzheimer's disease: current and future perspectives

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REVIEW

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Nonamyloid PET biomarkers and Alzheimer's disease: current and future perspectives

Lucas Porcello Schilling[1,2,3], Antoine Leuzy[1,2], Eduardo Rigon Zimmer[1,2,4], Serge Gauthier[2] & Pedro Rosa-Neto*[1,2]

ABSTRACT Recent advances in neurobiology and PET have helped redefine Alzheimer's disease (AD) as a dynamic pathophysiological process, clinically characterized by preclinical, mild cognitive impairment due to AD and dementia stages. Though a majority of PET studies conducted within these populations have to date focused on β-amyloid, various 'nonamyloid' radiopharmaceuticals exist for evaluating neurodegeneration, neuroinflammation and perturbations in neurotransmission across the spectrum of AD. Importantly, findings using such tracers have been shown to correlate with various clinical, cognitive and behavioral measures. In the context of a growing shift toward early diagnosis and symptomatic and disease-modifying clinical trials, nonamyloid PET radiotracers will prove of use, and, potentially, contribute to improved therapeutic prospects for AD.

KEYWORDS
- Alzheimer's disease
- neuroinflammation
- neuroreceptor systems
- nonamyloid imaging biomarkers • PET • tau

Alzheimer's disease (AD) is the leading cause of dementia [1] and is characterized by an insidious onset, progressive impairment in memory, attention and language and by the presence of neuropathological hallmarks, including the extracellular deposition of β-amyloid (Aβ) and the intracellular accumulation of hyperphosphorylated tau [2]. Criteria for the clinical diagnosis of AD were first put forth in 1984 by a work group jointly established by the National Institute of Neurological and Communicative Disorders and Stroke, and the Alzheimer's Disease and Related Disorders Association [3]. These criteria assumed that the clinical and neuropathological features of AD were related in a one-to-one manner, such that AD pathology was either present in an individual – producing dementia – or absent, in which case dementia, if present, was not due to AD [4].

Though useful and widely adopted, important advances in the characterization of AD along clinical and neuropathological lines, as well as in our ability to detect AD pathophysiology *in vivo* via biomarkers, led to a consensus position that the criteria should be revised [4]. These revisions, initiated by an International Working Group [5,6], were further elaborated by several National Institute of Aging and Alzheimer's Association working groups, resulting in novel diagnostic criteria addressing asymptomatic, 'preclinical' [7], mild cognitive impairment (MCI) due to AD [8] and AD dementia stages [9]. This revised conceptual framework posits a conceptual distinction between AD neuropathology and resulting clinical phenomenology. Specifically, AD has been conceptualized as a progressive pathophysiological process in which Aβ toxicity is believed to result in sequence of dynamic changes, including the accumulation of intracellular neurofibrillary tangles

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(NFTs), synaptic loss [10,11], neuroinflammatory processes [12] and disrupted neurotransmission [13]. Importantly, these changes are thought to accumulate during a protracted preclinical phase, with the acceleration of such changes marking the transition to MCI, the symptomatic predementia phase of AD.

Increasingly, quantitative imaging of functional and molecular processes in the living human brain has become possible using neuroimaging techniques, in particular PET (see **Figure 1**). Noninvasive in nature, PET allows for the quantification of brain biological processes by modeling interactions between short-lived radiopharmaceuticals and a biological process of interest, with sensitivity in the nano to pico molar (10^{-9}–10^{-12} M) range. While the majority of PET studies conducted in AD have to date focused on the characterization of amyloid fibrillary deposits, a growing number of 'nonamyloid radiopharmaceuticals are available for visualization and quantification of neurodegeneration (glucose metabolism and hyperphosphorylated tau), neuroinflammation (astrocytosis, microgliosis and phospholipase) and perturbations in neurotransmission (cholinergic, dopaminergic, serotonergic and others; see **Table 1** & **Figure 2**). This article aims to review the role of nonamyloid PET biomarkers in improving our understanding of AD neurobiology.

Pet biomarkers of neurodegeneration

• Imaging tau pathology

Misfolding and aggregation of hyperphosphorylated tau into NFTs is known to occupy a central mechanistic role in the pathogenesis of AD [49,50]. A growing body of evidence suggests that tau pathology is thought to spread to distant brain regions following a shift toward self-propagation in a prion-like manner, disrupting neuronal function and leading, ultimately, to neuronal loss and cognitive decline [51]. While increased cerebrospinal fluid (CSF) levels of tau have been shown to correlate with disease severity [52] – with the presence of tau in the CSF of AD patients thought to reflect neurodegeneration [53] – the use of CSF tau as a biomarker for AD remains controversial [54]. Moreover, in addition to the invasive nature of lumbar puncture, CSF measurements cannot provide topographic information, and are prone to variation across centers [55]. Noninvasive methods

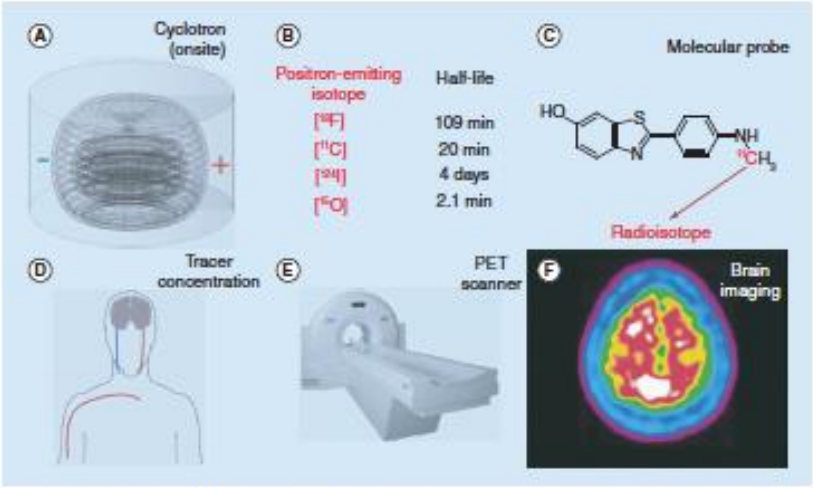

Figure 1. Steps involved in PET imaging. An on-site cyclotron (A) inside the radiochemistry facility produces short-lived positron-emitting isotopes (B) which, after quality control, are delivered as PET molecular probes (C) to hospital or research facilities. Molecular probes are injected intravenously at tracer concentrations (D) before PET scanning (E). During the scan, the PET camera records the distribution of radioactivity, which, following reconstruction, (F), can be analyzed and read by imaging experts or clinicians.
For color images please see online at http://www.futuremedicine.com/doi/full/10.2217/fnl.14.40

Table 1. Potential nonamyloid PET biomarkers for Alzheimer's disease.

Biological target	Radiopharmaceuticals	Findings	Interpretation	Ref.
Neurodegeneration				
Tau pathology	[^{18}F]T807	Increased	Increased formation of tau aggregates	[14]
	[^{18}F]T808	Increased		[15]
	[^{18}F]THK523	Increased		[16]
	[^{18}F]THK5105	Increased		[17]
	[^{18}F]THK5117	Increased		[17]
	[^{11}C]PBB3	Increased		[18]
		Increased		
Hexokinase activity	[^{18}F]FDG	Decreased	Decreased glucose metabolism	[19]
Neuroinflammation				
TSPO	[^{11}C]PK11195	Increased	Increased microglial activation	[20]
	[^{11}C]DAA1106	Increased	Increased microglial activation	[21]
	[^{18}F]FEDAA1106	Increased	Increased microglial activation	[22]
	[^{11}C]AC5216	Increased	Increased microglial activation	[23]
CB2	[^{11}C]A836339	Increased	Increased microglial activation	[24]
MAO-B	[^{11}C]L-deprenyl	Increased	Increased reactive astrocytosis	[25]
Phospholipase	1-[^{11}C]-AA	Increased	Increased phospholipase activity	[26]
Neurotransmission				
AChE activity	[^{11}C]PMP	Decreased	Decreased AChE activity	[27]
	[^{11}C]MP4A	Decreased		[28]
mAChR	[^{11}C] NMPB	Decreased	Decreased mAChR density	[29]
$mAChR_2$	[^{18}F]FP-TZTP	Decreased	Decreased $mAChR_2$ density	[30]
nAChRs	2-[^{18}F]A-85380	Decreased	Decreased nAChR density	[31]
	[^{11}C]nicotine	Decreased	Decreased nAChR density	[32]
nAChRs type $\alpha_4\beta_2$	[^{18}F]NCFHEB	Decreased	Decreased nAChRs type $\alpha_4\beta_2$ density	[33]
nAChRs type α_7	[^{11}C]CHIBA-1001	Decreased	Decreased nAChRs type α_7 density	[34]
VAChT	[^{18}F]FEOBV	Decreased	Decreased VAChT activity	[35]
D_1	[^{11}C]NNC756	Decreased	Decreased D_1 density	[36]
D_2	[^{11}C]raclopride	Decreased	Decreased D_2 density	[37]
$D_{2/3}$	[^{11}C]FLB457	Decreased	Decreased D_2/D_3 density	[38]
DAT	[^{11}C]β-CFT	Decreased	Decreased dopamine reuptake	[39]
VMAT2	[^{11}C]DTBZ	Decreased	Decreased VMAT2 activity	[40]
$5\text{-}HT_{2A}$	[^{18}F]setoperone	Decreased	Decreased $5\text{-}HT_{2A}$ receptor	[41,42]
	[^{18}F]altanserin	Decreased	densityDecreased $5\text{-}HT_{2A}$ receptor density	
$5\text{-}HT_{1A}$	[^{18}F]MPPF	Decreased	Decreased $5\text{-}HT_{1A}$ receptor density	[43]
$5\text{-}HT_4$	[^{11}C]SB207145	Increased	Increased $5\text{-}HT_4$ receptor density	[44]
NET	[^{18}F]FMeNER-D2	Decreased	Decreased NET density	[45]
H_1	[^{11}C]doxepine	Decreased	Decreased H_1 density	[46]
μ and κ opioid receptors	[^{18}F]fluoronaltrexone	Decreased	Decreased μ and κ opioid receptors densities	[47]
A_1	[^{11}C]MPDX	Decreased	Decreased A_1 density	[48]

5-HT: Serotonin receptor; A: Adenosine receptor; D: Dopamine receptor; DAT: Dopamine transporter; H: Histamine receptor; mAChR: mACh receptor; nAChR: Nicotinic acetylcholine receptor; NET: Norepinephrine transporter.

for quantification of tau pathology are therefore highly desirable, particularly given the increasing efforts directed towards the development of tau based therapeutics. In this context, several PET research groups have recently developed novel classes of compounds characterized by suitable kinetics and high affinity/selectivity for tau fibrils, including benzimidazole pyrimidine derivatives-[^{18}F]T-807 and [^{18}F]T-808-phenylquinoline derivatives-[^{18}F]THK-523, [^{18}F]THK-5105 and [^{18}F]THK-5117 and the benzothiazole derivative [^{11}C]PBB-3.

Following a screening of more than 900 compounds, several benzimidazole pyrimidine derivatives were identified as potential tau ligands, including [^{18}F]T-807 and [^{18}F]-T808. *In vitro*

autoradiography using human AD brain sections showed that [^{18}F]T-807 exhibits strong binding to NFTs, with a selectivity estimate of 29-fold for tau, relative to Aβ [14]. Moreover, comparison between double immunohistochemical staining of NFTs and Aβ on adjacent tissue sections and [^{18}F]T-807 autoradiography showed the colocalization of [^{18}F]T-807 binding with immunoreactive NFTs, but not with $A\beta_{1-42}$ plaques [56]. In the case of [^{18}F]T-808, *in vitro* autoradiographic-binding assays revealed high affinity and good selectivity for NFTs over Aβ [15], with *in vivo* assessment in wild-type mice and rats using micro-PET showing fast brain uptake followed by a rapid washout, suggesting low nonspecific binding [15], in line with *in vivo* finding obtained using [^{18}F]T-807 [56]. Finally, the first human brain images obtained using [^{18}F]T-807 revealed an elevated standardized uptake value ratio to the cerebellum in AD – as compared with subjects with MCI and healthy controls across temporal, parietal and frontal cortices, as well as in the hippocampus/entorhinal area, with the differential patterns of tracer accumulation observed in keeping with Braak staging [14].

Phenylquinoline derivatives exhibiting high affinity and selectivity for tau aggregates were pioneered by the Tohoku group, with [^{18}F]THK-523 [57] the first tau radiotracer candidate for PET. Initial *in vitro* binding assays showed [^{18}F]THK-523 to possess binding affinity for tau fibrils, with follow up autoradiographic work using AD medial temporal brain sections showing accumulation of [^{18}F]THK-523 in the hippocampal Sommer's sector, as well as in the pre- and pri-β layers of the entorhinal cortex. These findings were consistent with the density of paired helical filament (PHF)-tau deposition, as confirmed via immunohistochemistry [58]. Subsequent autoradiographic and histofluorescence studies showed that [^{18}F]THK-523 binding to NFTs colocalized with tau immonoreactivity, with no detectable binding to Aβ plaques [16]. Similar findings were obtained in transgenic mice (rTg4510), with [^{18}F]THK-523 found to colocalize with NFTs in tissue immunostained with tau antibodies, corroborating *in vivo* findings obtained using micro-PET. By contrast, no colocalization was noted in a model harboring human amyloid precursor protein and presenilin 1 mutations (APP/PS1), a model displaying

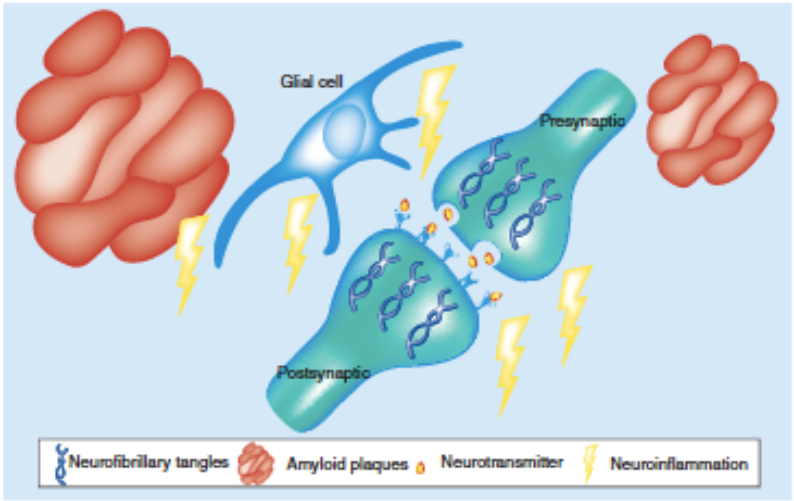

Figure 2. Alzheimer's disease pathophysiological events amenable to quantification using PET.
Hypothetical models of Alzheimer's disease suggest that the pathophysiological cascade of events culminating in dementia begins many years before detectable cognitive impairment. These models have provided the framework necessary to test various nonamyloid biomarkers addressing processes believed to be secondary to amyloid toxicity, including neurodegeneration, neuroinflammation and disruptions within neurotransmitter system.

amyloidosis in the absence of tau pathology, with micro-PET showing no difference in [18F]THK-523 between APP/PS1 mice and wild-type littermates [16]. More recent immunohistochemical and histofluorescence studies, however, suggest that [18F]THK-523 does not bind tau inclusions in non-AD tauopathies [59], with preliminary clinical data casting doubt on [18F]THK-523's future in both research and clinical settings owing to very high nonspecific WM binding [60].

Following optimization to improve specificity, second generation quinoline derivatives were introduced, in the form of [18F]THK-5105 and [18F]THK-5117. *In vitro* binding assays conducted using [18F]THK-5105 and [18F]THK-5117 showed high binding affinity to synthetic truncated tau (K18ΔK280) fibrils – comprising the four repeat regions (244-372) in the absence of lysine 280 (ΔK280) – with both tracers proving superior to [18F]THK-523. Further examination of the selective binding capacity of these compounds-performed using *in vitro* autoradiography and AD mesial temporal brain sections-showed elevated tracer accumulation within the parahippocampus and subiculum, with particularly high binding in the Sommer's sector of the hippocampus. These findings, confirmed with Gallyas–Braak staining and immunohistochemistry, were reduced among healthy controls [17]. Further assessment of [18F]THK-5105, conducted using AD hemibrain sections and [11C]PIB, showed dense accumulation of [18F]THK-5105 in tau rich areas – including the insula, inferior and middle temporal gyri, cingulate gyrus and hippocampus/parahippocampus-with the pattern of tracer retention corresponding to the known distribution of tau pathology but not to that of Aβ or areas showing elevated retention of [11C]PIB. Furthermore, biodistribution studies conducted in normal mice showed abundant and rapid brain uptake and fast clearance, with the kinetics of both tracers superior to those reported for [18F]THK-523 [17].

Following a screening of several fluorescent chemicals capable of binding to β-sheet conformations, a phenyl/pyridinyl-butadienyl-benzothiazoles/benzothiazoliums (PBBs) class of tau ligands was developed for visualization and quantitative assessment of diverse structural forms of phosphorylated tau. Two-photon microscopic data using the most promising of these probes, [11C]PBB3-a pyridinated PBB radiolabeled with carbon-11-provided strong evidence that [11C]PBB3 rapidly transits both the blood–brain barrier and neuronal plasma membranes, binding to intraneuronal tau inclusions. Moreover, accumulation of PBB3 in AT8-positive, NFT-like lesions in Tg mice expressing a single human four-repeat tau isoform with the P301S FTDP-17 mutation (PS19 line) was confirmed using *ex vivo* microscopy. Additionally, *in vitro* and *ex vitro* autoradiographic studies showed that [11C]PBB3 produced selective, high-contrast labeling of neuronal tau inclusions in the brain stem of PS19 mice. Similar findings were obtained using *in vitro* autoradiography and AD tissue, with marked radiolabeling of fibrillar aggregates in the frontal cortex, as well as the Sommer's sector and subiculum of the hippocampus. Finally, an exploratory clinical PET study using [11C]PBB3 in patients with probable AD revealed elevated tracer retention in lateral temporal and frontal cortices – consistent with distribution of tau pathology at Braak stage V/VI – with increasing standardized uptake value ratios correlating with decreased scores on the mini mental state examination (MMSE). In addition, a slight increase in [11C]PBB3 retention was noted around the hippocampus of a control subject who showed a decline in MMSE, consistent with Braak stage III/IV or earlier [18].

• Imaging brain glucose metabolism

[18F]FDG PET has long been used to investigate the neurodegenerative aspects of AD, with cerebral glucose metabolism taken as a proxy for neuronal activity [61], and as a marker of synaptic density [62]. In AD, metabolic deficits are found to follow a specific regional pattern, with declines in glucose metabolism noted in parietotemporal areas, precuneus [19], posterior cingulate cortex [63] and the medial temporal lobe (see **Figure 3**) [64]. These hypometabolic areas are noted to extend toward frontal association areas with progression of the disease, with relative preservation of the visual cortex, primary sensory motor cortices, basal ganglia, thalamus and cerebellum [19,64,65]. While this *in vivo* pattern of hypometabolism is found in the vast majority of clinically diagnosed AD patients and in over 85% pathologically confirmed cases [19], the extent and topography of hypometabolism has been found to vary across atypical focal cortical presentations of AD. Specifically, relative to typical AD, patients with posterior cortical atrophy have been shown to exhibit selective hypometabolism in occipito-parietal regions – as well as

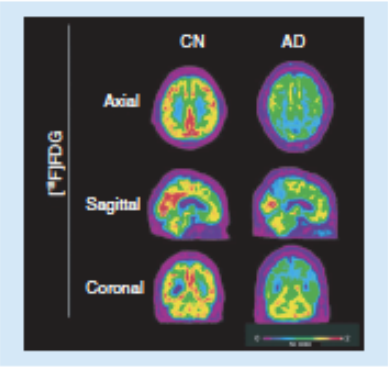

Figure 3. Representative [18F]FDG images in a cognitively normal subject and in a patient with Alzheimer's disease. Metabolic imaging with [18F]FDG PET in AD shows the characteristic pattern of reduced tracer emission, comprising bilateral parietal, temporal and posterior cingulate regions. In contrast, metabolism within these regions is preserved in the cognitively normal subject.
AD: Alzheimer's disease; CN: Cognitively normal.

in the frontal eye fields [66] – with logopenic primary progressive aphasia associated with disproportionate left temporoparietal hypometabolism [67]. Greater hypometabolism has likewise been noted in patients with early onset AD – with metabolic reductions among those with mild dementia comparable to that seen in late onset cases with severe dementia [68] – consistent with studies showing more rapid progression among patients with early onset AD [69] and, potentially, with cognitive reserve theory [70].

Among patients with MCI, metabolic abnormalities are usually noted in brain areas typically affected in AD [71], albeit of inferior magnitude [72–74], with the anterior hippocampal formation of particular value for differentiating subjects with MCI from healthy controls [73]. In the context of conversion studies, MCI subjects presenting a more pronounced or 'AD-like' pattern have been found to decline to AD at higher rates [75,76], with reductions in glucose metabolism shown to predict future AD with accuracies in the range of 75–100% [77,78]. In addition, [18F]FDG has proven of use in differentiating progressive from nonprogressive MCI, particularly when combined with memory scores [79], with progressors showing the typical AD functional pattern comprising hypometabolism in the parietal and posterior cingulate cortices, and more severe memory impairment [79,80]. Among nonprogressors, memory impairment was found to be less severe, with hypometabolism confined to the dorsolateral frontal cortex, consisted with this regions role in episodic memory processes such as encoding and retrieval [81,82].

[18F]FDG-PET has likewise been used to study the progression to MCI and AD among cognitively normal older individuals, and has been shown to predict cognitive decline within this population with an accuracy approaching 80% [83,84]. In a clinicopathological study incorporating longitudinal [18F]FDG among cognitively normal individuals followed through MCI to pathologically confirmed AD, progressive reductions in glucose metabolism were noted years in advance of clinical symptoms, with reductions in the hippocampus preceding declines in cortical regions [85]. Similar functional changes have been observed in cognitively normal individuals homozygous for the *APOE* ε4 allele [79,86] – a susceptibility gene-asymptomatic carriers of mutations causative for early onset familial AD [77–78,87], and among those with a maternal family history of AD, as compared with those with a paternal history or no family history of AD [88]. Finally, [18F]FDG may prove of use in the characterization of a subset of patients exhibiting neurodegeneration in the absence of Aβ deposition [20,89]. Placed in the category of 'suspected nonamyloid pathophysiology' (SNAP), these patients have led to the hypothesis that the onset of neurodegeneration in AD may not depend of the accumulation of Aβ [90].

PET biomarkers for neuroinflammation

• Imaging microglial activation

AD is associated with neuroinflammatory processes, with the key cellular event signaling its presence the accumulation of reactive microglia in areas affected by neurodegeneration [91]. Observed in both sporadic and familial forms of AD, significant microglial activation appears to occur early on in the disease course, with a significant increase in the fractional area of reactive microglia accompanying the formation of neuritic plaques comprising fibrillary Aβ [21–22,92–93]. *In vivo* quantification of microglial activation has been achieved using [11C]PK11195, a specific ligand for TSPO, formerly called the peripheral benzodiazepine receptor [23]. While early work using racemic [11C]PK11195 was negative [94] – likely due to the relatively high

level of nonspecific binding – more recent studies using the R-isomer have shown increased binding of [^{11}C]-(R)-PK11195 in the entorhinal, temporoparietal and cingulate cortices of AD patients in comparison to healthy control subjects [21]. Moreover, high microglial activation has been observed in [^{11}C]PIB+ AD patients, with an inverse correlation found between cortical microglial activation and cognition [95]. Novel approaches aiming to augment the sensitivity of [^{11}C]-(R)-PK11195 signal modelling may be required; in this respect, a recent study has shown that the inclusion of a vascular component results in an amplified signal in patients with AD [96].

Additional ligands targeting TSPO have been developed aiming for improved pharmacokinetics and specificity. For example, PET imaging with [^{11}C]DAA1106 showed robust binding in AD patients, compared with healthy controls [97], with [^{18}F]FEDAA1106 or [^{11}C]AC5216 showing promising results in preclinical PET studies in AD-like transgenic models [98,99]. Further, a recent clinical study showed that while increasing binding of [^{18}F]FEDAA1106 was not seen in AD, widespread increases were noted in MCI, when compared with healthy controls, with these values predictive of conversion to AD dementia within a 5-year follow-up period [24]. These findings contrast with those obtained using [^{18}F]FEDAA1106 – a novel TSPO ligand with *in vitro* affinity superior to that of [^{11}C]DAA1106 [100] – with no statistically significant differences in binding between healthy controls and AD patients, possibly due to the inability to separate specific and nonspecific signals *in vivo* [101]. Though not yet tested in AD patients, [^{11}C]AC5216 has shown promising pharmacokinetic properties and higher affinity than [^{11}C]PK11195 in healthy subjects [102]. However, potential clinical utility of TSPO ligands is undermined by the rs6971 polymorphism in the *TSPO* gene, which confers lower uptake in carriers (~30%) in comparison to noncarriers [25]. Finally, growing interest in the endocannabinoid system has identified the cannabinoid receptor type 2 (CB2) as a marker of microglial activation [103]. Specifically, preclinical studies show upregulation of CB2 – as indexed by [^{11}C]A-836339-in a transgenic model displaying cerebral amyloidosis [26].

• Imaging reactive astrocytosis

Increased expression of glial fibrillary acidic and astroglial S100B proteins are typically observed in AD postmortem tissue, indicating an increased number of reactive astrocytes [104]. PET studies using [^{11}C]DED – a ligand with high affinity/specificity for monoamine oxidase B, an enzyme expressed primarily on the mitochondrial membrane of reactive astrocytes [105,106] – have shown elevated binding in patients with MCI, pointing to astrocytosis as an early event in AD [107].

• Imaging phospholipase activity

Microglia-derived inflammatory cytokines are capable of binding to astrocytic cytokine receptors coupled to $cPLA_2$, and $sPLA_2$ [108], Ca^{2+}-dependent enzymes whose activation initiates the hydrolysis of esterified arachidonic acid (AA) from membrane phospholipids [109]. Release of nitric oxide can likewise promote membrane-based AA hydrolysis via the role of $cPLA_2$ *vis à vis* ionotropic glutamate receptor mediated increases in intracellular Ca^{2+} concentrations [110–112]. Indeed, increased cytokine levels have been noted in AD [113], as have increased expression levels of both $cPLA_2$ and $sPLA_2$, CSF levels of AA metabolites-isoprostane and isofluorane-[114], and glutamatergic markers [27,28,115], suggesting that AD is associated with increased AA metabolism. Preliminary results obtained using 1-[^{11}C]-AA support this hypothesis, showing elevated incorporation coefficients in neocortical areas shown to have high densities of senile (neuritic) plaques with activated microglia. To the extent that the elevated binding of 1-[^{11}C]-AA represents the upregulation of AA metabolism secondary to neuroinflammation, PET with 1-[^{11}C]-AA may be used to examine neuroinflammation in patients with AD [109].

PET biomarkers for neurotransmission

• Imaging cholinergic neurotransmission

Degeneration of the cholinergic system is a well-established biochemical and histopathological feature of AD [116], with reduction in levels of acetylcholine (ACh) and AChE – the most important enzyme mediating hydrolysis of ACh in the human brain – consistently described [117,118]. Indeed, radiolabeled analogs of ACh – such as [^{11}C]PMP and [^{11}C]MP4A, themselves substrates for AChE – can be used for measuring and imaging its activity *in vivo* [119,120].

PET studies using [^{11}C]PMP and [^{11}C]MP4A have consistently found reduced cortical activity in AD, with the severity of such reductions greatest in the temporal cortex [121–124]. In a study

examining the association between [^{11}C]MP4A imaging and *APOE* ε4 genotype in patients with AD, AChE was found to be lower in ε4 noncarriers [29], with the relative preservation of cortical AChE activity in β4 carriers possibly due to its preserved cellular expression or as a result of AChE activity in amyloid plaques [30]. In AD, AChE activity, as indexed by [^{11}C]PMP, has been shown to associate significantly with performance on executive measures, as opposed to tests reflecting episodic memory [31]. Reduced AChE activity has also been reported in MCI [124], particularly in those subjects who later convert to AD [32], suggesting that low AChE activity may be a marker of prodromal AD. [^{11}C]PMP and [^{11}C]MP4A have likewise been used to examine the effects of currently available cholinesterase inhibitors [118] in AD, with standard clinical doses producing a 30–40% inhibition of AChE [31,125–126].

ACh exerts its effects on the CNS via binding at the ligand-gated nicotinic ACh receptors (nAChRs) – consisting of five subunits, variously comprising α2–α10 and β2-β4 and the G-protein coupled muscarinic receptors (mAChRs), proteins known to play an important role in the neuronal circuitry underlying attention, learning and memory. To date, there have been few PET studies targeting mAChRs in AD. Using [^{11}C]NMPB, a nonselective mAChR ligand, no changes were observed in patients with AD [127]. Higher binding was, however, shown in AD *APOE* ε4 carriers using [^{18}F]FP-TZTP, a tracer selective for the mAChR type 2 [128]. In the case of nAChRs, studies have been more numerous, with three general compound classes of radiopharmaceuticals developed for use with PET: nicotine and its derivatives, 3-pyridyl ether derivatives-including 2-[^{18}F]A-85380 – and derivatives of epibatidine [129].

PET studies using [^{11}C]nicotine have shown decreased availability of nAChRs in AD [33], with such reductions shown to correlate significantly with performance decrements on measures of executive functioning, in particular, attention [130]. When measured before and after treatment with rivastigmine – an AChE inhibitor – [^{11}C]nicotine binding sites were found to be significantly increased, with this increase found to correlate with improved performance on an attentional task at 12-month follow-up [126,131]. However [^{11}C]nicotine results are contaminated by cerebral blood flow effects (see review, [34]). In the case of 2-[^{18}F]A-85380, a tracer specific to the α4β2 nAChR subtype, results have proven inconsistent, with reports of no differences between patients with AD and age-matched healthy controls [132] and reduced availability of α4β2 receptors in both MCI and AD [35]. Owing to the lengthy acquisition time required for studies using 2-[^{18}F]A-85380 (7–8 h), additional α4β2 nAChRs radioligands have been developed, including [^{18}F]NCFHEB. Preclinical work in a porcine model has established that in comparison to 2-[^{18}F]A-85380, [^{18}F]NCFHEB enantiomers possess superior kinetics and shorter acquisition times [133].

A recent approach has involved the development of PET tracers selective for the α7 nAChR subtype. Along with $\alpha_4\beta_2$, the α_7 subtype is the most abundant nAChR in the human brain and, is known to occupy a key role in neuronal plasticity, sensory gating and memory. In contrast to α4β2, however – whose numbers are decreased in AD – the α_7 receptor population has been shown to remain largely intact in AD, and as such, stands as a potential target for novel therapeutics [134]. An interesting approach is to develop PET tracers selective for α7 nAChRs, as this receptor subtype may have broad interactions with several neurotransmitter systems and pathologic processes in AD [135]. Recently, the results of PET studies have suggested that [^{11}C]CHIBA-1001 may be a suitable radioligand for imaging α7 nAChRs in the human brain, possessing desirable prerequisites for being considered adequate for PET imaging [36]. Finally, several *in vitro*/*in vivo* studies [37–40] using the vesamicol derivative [^{18}F]FEOBV – which binds VAChT – suggest this tracer to be a promising marker of brain VAChT, sensitive to subtle disruptions of the cholinergic system [38].

• Imaging dopaminergic neurotransmission

Various aspects of the dopaminergic system can be assessed using PET, including synthesis, receptor densities, uptake system, vesicular transporter and neurotransmission release. In contrast to other neurodegenerative diseases, studies using [^{18}F]fluorodopa in AD have shown dopamine synthesis to be preserved, even in the presence of mild 'parkinsonian' rigidity [136]. Relative to controls, reduced striatal uptake of the dopamine D1 receptor antagonist [^{11}C]NNC756 has been show in AD patients, in contrast to the absence of significant declines in striatal dopamine D2 receptors, as assessed using [^{11}C]raclopride. Moreover, findings with either

tracer were not found to correlate with MMSE or extrapyramidal symptoms, measured using the Unified Parkinson's Disease Rating Scale [137]. Use of [^{11}C]raclopride in patients with more advanced AD, however, has shown a correlation between decreased binding and scores obtained on the Behavioral Pathology in AD Frequency Weighted Severity Scale [138]. Using the dopamine D2/D3 receptor antagonist [^{11}C]FLB457, declines in hippocampal and temporal cortex D2 receptors have been observed in AD, with reductions in the right hippocampus shown to associate significantly with verbal memory performance and declarative naming [139]. Further, using [^{11}C]β-CFT, a cocaine analog, striatal dopamine reuptake was found to be reduced in AD patients, with the severity of such reductions found to correlate with extrapyramidal symptom severity [140]. Finally, several PET studies using [^{11}C]DTBZ – a reliable marker of dopaminergic presynaptic integrity owing to its affinity for the vesicular monoamine transporter 2 – support its use to facilitate in the differential diagnosis of AD, dementia with Lewy Bodies and Parkinson's disease [141,142], with decreased binding of [^{11}C]DTBZ in patients with AD a potential marker for coexisting subclinical dementia with Lewy bodies pathology [143].

• Imaging serotoninergic neurotransmission

Widely involved on pathophysiology of mood and anxiety, disruption of the serotonergic system has been reported to closely parallel the occurrence of neuropsychiatric features in AD [144]. While the loss of serotonergic neurons within the raphe nuclei [145], as well as dysfunction of isocortical and allocortical serotonin nerve terminals [146–148] have been reported in AD, results have proven contradictory with respect to the correlation between such findings and neuropsychiatric symptoms [149,150]. Despite numerous lines of evidence supporting this serotonin (5-HT) deficiency theory [41–44,151], *in vivo* studies using PET have largely focused on the link between 5-HT receptors and cognitive impairment in AD [152].

Using [^{18}F]setoperone, a PET ligand specific to the 5HT$_{2A}$ receptor, significant declines were noted in the cortical 5-HT$_{2A}$ population, with maxima in the frontal, parietal, temporal and occipital cortices, as well as in the parieto-temporal carrefour [153]. In a similar study using [^{18}F]altanserin, likewise a ligand for the 5HT$_{2A}$ receptor, decreased binding was noted in several brain regions, including the prefrontal and sensorimotor cortices, as well as the in anterior cingulate. Moreover, no correlation was seen between ligand binding and dementia severity [154], indexed using the MMSE [45]. In a study measuring 5HT$_{1A}$ receptor densities using [^{18}F] MPPF and PET in patients with AD significantly decreased receptor densities were noted in the hippocampus and the raphe nuclei [155]. Reduction in hippocampal binding was significantly greater when hippocampal volume loss was accounted for, with a strong correlation found between such declines and clinical severity, measured with the MMSE [155]. Finally, a recent study using [^{11}C]SB207145, a novel radioligand selective for the 5-HT$_4$ receptor, showed that while no differences existed between patients with AD and healthy controls when AD was defined on clinical grounds, increased binding was noted in amyloid positive individuals. The authors suggest that upregulation of 5-HT$_4$ may begin in preclinical AD, possibly in an attempt to counteract the accumulation of Aβ [156].

• Imaging other neurotransmitters

Numerous postmortem studies indicate that cell numbers within the locus coeruleus (LC) – the primary source of norepinephrine (NE) in the brain – are significantly decreased in AD, as are levels of NET [46,157–158]. In a series of autoradiographic experiments using (S,S)-[^{18}F] FMeNER-D, a novel PET ligand selective for NET, significant declines in NET densities were noted in the LC and thalamus of AD tissue sections, as compared with healthy controls [159]. These results suggest that NET levels stand as a potential biomarker for AD, and that the thalamus – as opposed to the LC, whose small size is problematic given the spatial resolution of current diagnostic PET scanners – may prove a suitable target for future *in vivo* PET studies using (S,S)-[^{18}F]FMeNER-D.

Histaminergic receptors have been shown a role an important role in the modulation of learning, memory [160] and attention [161], and are implicated in antiapoptopic pathyways [161] as well as in the mediation of neuroprotective effects at the level of the hippocampus [162]. In this respect, a PET study using [^{11}C]doxepine, an H1 receptor ligand, showed reduced binding in temporal and frontal brain areas in AD patients, with receptor binding correlating with clinical severity, as measured using the MMSE [47]. These findings suggest that histaminergic

disruption may contribute to the cognitive deficits seen in AD.

Interest likewise exists in the use of PET for studying the opioidergic system in AD given the putative modulatory role of the endogenous opioid system in behavior and cognition [163], the observation that high doses of the opiate receptor antagonist naloxone produce cognitive impairment in normal subjects [164,165] and postmortem studies showing declines in opiate receptors in AD [166,167]. *In vivo* work aiming to further clarify the role of the opioidergic system in AD has shown declines in μ and -k receptor subtypes using [^{18}F]fluoronaltrexone, a μ and -k receptor antagonist, with significant declines noted in the parietal, frontal and limbic cortices, but not in the temporal cortex [168].

Finally, decreased density of the adenosine A_1 receptor has been noted in postmortem autoradiographic and pathological studies using hippocampal AD tissue [48,169–171]. Citing the observation that despite considerable neuronal loss within the medial temporal cortex, patients with AD do no invariably exhibit medial temporal cortex hypometabolism following [^{18}F]FDG PET [64,172,173], Fukumitsu *et al.* [174] argued that [^{11}C]MPDX, a tracer with affinity for the adenosine A_1 receptor, may prove of use as a diagnostic biomarker in AD. An *in vivo* study using [^{18}F]FDG PET and [^{11}C]MPDX in a small sample of AD patients and healthy controls showed that while decreased retention of [^{11}C]MPDX was observed in the temporal and medial temporal cortices, declines in [^{18}F]FDG were noted only in the temporal cortex. As such, the authors proposed that [^{11}C]MPDX, but not [^{18}F]FDG, may prove sensitive to disrupted neuronal integrity within the performant path and its terminal, owing to it being a site characterized by high density of adenosine A_1 receptors [175].

Limitations of nonamyloid PET biomarkers for AD

A majority of PET radiotracers targeting nonamyloid processes are labeled with carbon-11 (11-C), which stands as a limitation owing to the short (~20 min) half-life of this isotope. Production of 11-C radioligands is therefore limited to imaging centers possessing an onsite cyclotron and a radiochemistry department with the requisite expertise, making the cost of studies prohibitive for routine clinical use. Though fluorine-18 radiopharmaceuticals have been introduced-with a half-life of approximately 110 min allowing for centralized production and regional distribution-only [^{18}F]FDG is currently approved for clinical use. In the case of TSPO ligands, their clinical utility may further be hampered by differential binding affinity among carriers of a common single-nucleotide polymorphism (rs6971) in exon 4 of the *TSPO* gene [176,177]. The issue of specificity for AD likewise stands as a limitation owing to the presence of neuroinflammation, deposition of hyperphosphorylated tau, dysregulation of neurotransmitter systems and hypometabolic changes across a wide range of neurodegenerative diseases (for review, see [30,178–180]).

Conclusion

Though the field retains a focus on the investigation of Aβ deposition owing to current explanatory models, a growing body of studies using functional and molecular PET imaging suggest that the clinical evolution of AD is driven by downstream processes, with findings using radiotracers for hyperphosphorylated tau, cerebral glucose metabolism, neuroinflammation as well as for monoaminergic and related transmitter systems, shown to correlate with various cognitive and clinico-behavioral measures. Though much work remains, implementation of multitracer approaches using the biomarkers here reviewed may facilitate the refinement of current models, allowing for an improved understanding of AD pathophysiology.

Future perspective

On the basis of recent biomarker studies, AD pathophysiology is thought to precede the onset of clinical symptoms by up to a decade or more. As such the field has begun to shift toward the early diagnosis of AD, recognizing that a relatively broad window of opportunity exists, potentially, for intervention with novel disease modifying therapies. Though none have yet to demonstrate efficacy in Phase III studies, various strategies targeting primarily amyloid and tau are under development. As such, multitracer approaches utilizing, for instance, [^{18}F]FDG and tau ligands, will be required in both symptomatic and disease-modifying trials in order to determine tau engagement and treatment response. Similarly, neuroinflammatory molecular imaging can similarly contribute to anti-inflammatory interventions. Finally, on the basis of the vast literature reporting the effects of psychiatric drug, tracers for neurotransmission may contribute to

the development of improved therapeutic prospects addressing neuropsychiatric features in AD as well as the refinement of current treatment protocols and improved study designs.

Financial & competing interests disclosure

This work was supported by the Canadian Institutes of Health Research (CIHR: MOP-11-51-31 and MOP 10-27-52), Alzheimer's Association (NIRG-12-259245), Fonds de Recherche du Québec – Santé (FRQS: Chercheur Boursier), the Allan Tiffin Trust (Infrastructure), the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq, Brazil), Fundação de Amparo a Pesquisa do Rio Grande do Sul (Fapergs, Brazil), and INCT for Excitotoxicity and Neuroprotection/CNPq. This research was also partially supported by Fonds d'innovation Pfizer-FRQS sur la maladie d'Alzheimer et les maladies apparentées – Volet 2 to P Rosa-Neto. S Gauthier and P Rosa-Neto are members of the CIHR Canadian Consortium of Neurodegeneration in Ageing. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

No writing assistance was utilized in the production of this manuscript.

EXECUTIVE SUMMARY

PET biomarkers of neurodegeneration

- Imaging tau pathology:
  - PET studies and related *in vitro* findings highlight the suitable kinetics and high affinity/selectivity for tau fibrils of novel tau radiotracers; however, validation studies are necessary.
- Imaging brain glucose metabolism:
  - Though declines in [^{18}F]FDG adhere to a specific topographic distribution in Alzheimer's disease (AD), focal [^{18}F]FDG patterns have been noted in atypical presentations. Greater hypometabolism has likewise been noted in patients with early onset AD;
  - [^{18}F]FDG hypometabolism has been noted in patients at various points along the AD continuum and in those at risk for AD. [^{18}F]FDG may likewise prove of use in terms of characterizing patients with suspected non-AD pathophysiology (SNAP).

PET biomarkers for neuroinflammation

- Imaging microglial activation:
  - The clinical utility of TSPO PET ligands are potentially limited owing to a polymorphism in the *TSPO* gene conferring low uptake in carriers.
- Imaging reactive astrocytosis:
  - Increased binding of [^{11}C]DED has been noted in patients with AD and mild cognitive impairment.
- Imaging phospholipase activity:
  - Elevated metabolism of arachidonic acid (AA)-indexed by 1-[^{11}C]-A- has been noted in AD.

PET biomarkers for neurotransmission

- Imaging cholinergic neurotransmission:
  - Reduced uptake of radiolabeled acetylcholine analogues, nicotine and its derivatives and 3-pyridyl ether/epibatidine derivatives are seen in patients with mild cognitive impairment and AD.
- Imaging dopaminergic neurotransmission:
  - [^{18}F]fluorodopa PET show that the synthesis and storage of dopamine (DA) is preserved in AD. [^{11}C]NNC756 shows declines in DA D1 receptor availability. DA, as indexed using [^{11}C]β-CFT, is reduced in the striatum;
  - Hippocampal and temporal cortex DA D2 receptors have been shown to be reduced using [^{11}C]FLB457, in the right hippocampal findings associated with memory scores;
  - In AD patients, decreased binding of [^{11}C]DTBZ, a ligand with affinity for the vesicular monoamine transporter 2, may be a marker of Lewy Body pathology.

EXECUTIVE SUMMARY (CONT.)

- Imaging serotoninergic neurotransmission:
  - PET studies using [^{18}F]setoperone and [^{18}F]altanserin show declines in neocortical $5HT_{2A}$ receptors;
  - Using [^{18}F]MPPF, decreased $5HT_{1A}$ receptor densities were noted in the hippocampus and raphe nuclei of patients with AD, with hippocampal binding correlating with clinical severity;
  - Findings with the 5-HT_6 ligand [^{11}C]SB207145 suggest that upregulation of 5-HT_6 may characterize preclinical AD.
- Imaging other neurotransmitters:
  - Autoradiographic studies using (S,S)-[^{18}F]FMeNER-D, a PET ligand selective for the norepinephrine transporter, have shown declines in norepinephrine transporter densities in the locus coeruleus and thalamus of AD tissue;
  - Binding of [^{11}C]doxepine, a histamine H1 receptor PET ligand, has been found to be reduced in temporal and the frontal brain areas in AD patients, with receptor binding correlating with clinical severity;
- Imaging other neurotransmitters:
  - *In vivo* studies in AD using [^{18}F]fluoronaltrexone, a μ and -k opioid receptor antagonist, have shown declines in parietal, frontal and limbic cortices;
  - Decreased density of the adenosine A_1 receptor has been noted in postmortem studies using hippocampal AD tissue. [^{11}C]MPDX, a tracer with affinity for the adenosine A_1 receptor, may prove sensitive to the integrity of the perforant path and its terminal zone.

References

Papers of special note have been highlighted as: * of interest; ** of considerable interest.

1 Brookmeyer R, Johnson E, Ziegler-Graham K, Arrighi HM. Forecasting the global burden of Alzheimer's disease. *Alzheimers Dement.* 3(3), 186–191 (2007).

2 Duyckaerts C, Delatour B, Potier MC. Classification and basic pathology of Alzheimer disease. *Acta Neuropathol.* 118(1), 5–36 (2009).

3 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* 34(7), 939–944 (1984).

4 Jack CR, Jr., Albert MS, Knopman DS *et al.* Introduction to the recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. *Alzheimers Dement.* 7(3), 257–262 (2011).

5 Dubois B, Feldman HH, Jacova C *et al.* Research criteria for the diagnosis of Alzheimer's disease: revising the NINCDS-ADRDA criteria. *Lancet Neurol.* 6(8), 734–746 (2007).

6 Dubois B, Feldman HH, Jacova C *et al.* Revising the definition of Alzheimer's disease: a new lexicon. *Lancet Neurol.* 9(11), 1118–1127 (2010).

7 Sperling RA, Aisen PS, Beckett LA *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.* 7(3), 280–292 (2011).

8 Albert MS, Dekosky ST, Dickson D *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.* 7(3), 270–279 (2011).

9 Mckhann GM, Knopman DS, Chertkow H *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.* 7(3), 263–269 (2011).

10 Jack CR, Jr., Knopman DS, Jagust WJ *et al.* Hypothetical model of dynamic biomarkers of the Alzheimer's pathological cascade. *Lancet Neurol.* 9(1), 119–128 (2010).

11 Jack CR, Jr., Knopman DS, Jagust WJ *et al.* Tracking pathophysiological processes in Alzheimer's disease: an updated hypothetical model of dynamic biomarkers. *Lancet Neurol.* 12(2), 207–216 (2013).

12 Mcgeer PL, Mcgeer EG. The amyloid cascade-inflammatory hypothesis of Alzheimer disease: implications for therapy. *Acta Neuropathol.* 126(4), 479–497 (2013).

13 Govoni S, Mura E, Preda S *et al.* Dangerous liaisons between beta-amyloid and cholinergic neurotransmission. *Curr. Pharm. Des.* 20(15), 2525–2538 (2014).

14 Chien DT, Bahri S, Szardenings AK *et al.* Early clinical PET imaging results with the novel PHF-tau radioligand [F-18]-T807. *J. Alzheimers Dis.* 34(2), 457–468 (2013).

15 Zhang W, Arteaga J, Cashion DK *et al.* A highly selective and specific PET tracer for imaging of tau pathologies. *J. Alzheimers Dis.* 31(3), 601–612 (2012).

16 Fodero-Tavoletti MT, Okamura N, Furumoto S *et al.* ^{18}F-THK523: a novel *in vivo* tau imaging ligand for Alzheimer's disease. *Brain* 134(Pt 4), 1089–1100 (2011).

17 Okamura N, Furumoto S, Harada R *et al.* Novel ^{18}F-labeled arylquinoline derivatives for noninvasive imaging of tau pathology in Alzheimer Disease. *J. Nucl. Med.* 54(8), 1420–1427 (2013).

18 Maruyama M, Shimada H, Suhara T *et al.* Imaging of tau pathology in a tauopathy mouse model and in Alzheimer patients compared with normal controls. *Neuron* 79(6), 1094–1108 (2013).

- **Recent work with the novel tau ligand [^{11}C] PBB3 reporting *in vivo* findings consistent with the temporospatial spread of hyperphosphorylated tau in Alzheimer's disease (AD).**

19 Silverman DH, Small GW, Chang CY *et al.* Positron emission tomography in evaluation of dementia: Regional brain metabolism and long-term outcome. *JAMA* 286(17), 2120–2127 (2001).

20 Jack CR, Jr., Knopman DS, Weigand SD *et al.* An operational approach to National Institute on Aging-Alzheimer's Association criteria for preclinical Alzheimer disease. *Ann. Neurol.* 71(6), 765–775 (2012).

- **Describes a subset of patients, dubbed 'suspected non-Alzheimer's disease pathophysiology' (SNAP) both with and without subtle cognitive impairment, characterized by neurodegeneration in the absence of β-amyloid deposition.**

21 Cagnin A, Brooks DJ, Kennedy AM *et al.* *In-vivo* measurement of activated microglia in dementia. *Lancet* 358(9280), 461–467 (2001).

22 Versijpt JJ, Dumont F, Van Laere KJ *et al.* Assessment of neuroinflammation and microglial activation in Alzheimer's disease with radiolabelled PK11195 and single photon emission computed tomography. A pilot study. *Eur. Neurol.* 50(1), 39–47 (2003).

23 Papadopoulos V, Baraldi M, Guilarte TR *et al.* Translocator protein (18kDa): new nomenclature for the peripheral-type benzodiazepine receptor based on its structure and molecular function. *Trends Pharmacol. Sci.* 27(8), 402–409 (2006).

24 Yasuno F, Kosaka J, Ota M *et al.* Increased binding of peripheral benzodiazepine receptor in mild cognitive impairment-dementia converters measured by positron emission tomography with [(1)(1)C]DAA1106. *Psychiatry Res.* 203(1), 67–74 (2012).

25 Yoder KK, Nho K, Risacher SL, Kim S, Shen L, Saykin AJ. Influence of TSPO genotype on ^{11}C-PBR28 standardized uptake values. *J. Nucl. Med.* 54(8), 1320–1322 (2013).

- **Recent work showing that the second-generation translocator protein (TSPO) ligand [^{11}C]-PBR28 is sensitive to TSPO genotype.**

26 Benito C, Tolon RM, Pazos MR, Nunez E, Castillo AI, Romero J. Cannabinoid CB2 receptors in human brain inflammation. *Br. J. Pharmacol.* 153(2), 277–285 (2008).

27 Mattson MP, Chan SL. Neuronal and glial calcium signaling in Alzheimer's disease. *Cell Calcium* 34(4–5), 385–397 (2003).

28 Lehtonen JY, Holopainen JM, Kinnunen PK. Activation of phospholipase A2 by amyloid beta-peptides *in vitro*. *Biochemistry* 35(29), 9407–9414 (1996).

29 Eggers C, Herholz K, Kalbe E, Heiss WD. Cortical acetylcholine esterase activity and ApoE4-genotype in Alzheimer disease. *Neurosci. Lett.* 408(1), 46–50 (2006).

30 Nordberg A, Rinne JO, Kadir A, Langstrom B. The use of PET in Alzheimer disease. Nature reviews. *Neurology* 6(2), 78–87 (2010).

31 Bohnen NI, Kaufer DI, Hendrickson R *et al.* Degree of inhibition of cortical acetyl-cholinesterase activity and cognitive effects by donepezil treatment in Alzheimer's disease. *J. Neurol. Neurosurg. Psychiatry* 76(3), 315–319 (2005).

32 Herholz K, Weisenbach S, Kalbe E, Diederich NJ, Heiss WD. Cerebral acetylcholine esterase activity in mild cognitive impairment. *Neuroreport* 16(13), 1431–1434 (2005).

33 Nordberg A, Lundqvist H, Hartvig P, Lilja A, Langstrom B. Kinetic-analysis of regional (S) (-)C-11-nicotine binding in normal and alzheimer brains - *in-vivo* assessment using positron emission tomography. *Alzheimer Dis. Assoc. Disord.* 9(1), 21–27 (1995).

34 Bohnen NI, Frey KA. Imaging of cholinergic and monoaminergic neurochemical changes in neurodegenerative disorders. *Mol. Imaging Biol.* 9(4), 243–257 (2007).

35 Sabri O, Kendziorra K, Wolf H, Gertz HJ, Brust P. Acetylcholine receptors in dementia and mild cognitive impairment. *Eur. J. Nucl. Med. Mol. Imaging* 35(Suppl 1), S30–S45 (2008).

36 Toyohara J, Sakata M, Wu J *et al.* Preclinical and the first clinical studies on [^{11}C] CHIBA-1001 for mapping alpha7 nicotinic receptors by positron emission tomography. *Ann. Nucl. Med.* 23(3), 301–309 (2009).

37 Parent M, Bedard MA, Aliaga A *et al.* PET imaging of cholinergic deficits in rats using [^{18}F]fluoroethoxybenzovesamicol ([^{18}F] FEOBV). *Neuroimage* 62(1), 555–561 (2012).

38 Parent MJ, Bedard MA, Aliaga A *et al.* Cholinergic depletion in Alzheimer's disease shown by [(18) F]FEOBV Autoradiography. *Int. J. Mol. Imaging* 2013 , 205045 (2013).

39 Parent MJ, Cyr M, Aliaga A *et al.* Concordance between *in vivo* and postmortem measurements of cholinergic denervation in rats using PET with [18F] FEOBV and choline acetyltransferase immunochemistry. *EJNMMI Res.* 3(1), 70 (2013).

40 Cyr M, Parent MJ, Mechawar N *et al.* PET imaging with [(1)(8)F]fluoroethoxy-benzovesamicol ([(1)(8)F]FEOBV) following selective lesion of cholinergic pedunculopontine tegmental neurons in rat. *Nucl. Med. Biol.* 41(1), 96–101 (2014).

41 Assal F, Alarcon M, Solomon EC, Masterman D, Geschwind DH, Cummings JL. Association of the serotonin transporter and receptor gene polymorphisms in neuropsychiatric symptoms in Alzheimer disease. *Arch. Neurol.* 61(8), 1249–1253 (2004).

42 Curcio CA, Kemper T. Nucleus raphe dorsalis in dementia of the Alzheimer type: neurofibrillary changes and neuronal packing density. *J. Neuropathol. Exp. Neurol.* 43(4), 359–368 (1984).

43 Nyth AL, Gottfries CG. The clinical efficacy of citalopram in treatment of emotional disturbances in dementia disorders. A Nordic multicentre study. *Br. J. Psychiatry* 157, 894–901 (1990).

44 Petracca G, Teson A, Chemerinski E, Leiguarda R, Starkstein SE. A double-blind placebo-controlled study of clomipramine in depressed patients with Alzheimer's disease. *J. Neuropsychiatry Clin. Neurosci.* 8(3), 270–275 (1996).

45 Folstein MF, Folstein SE, Mchugh PR. "Mini-mental state". A practical method for grading the cognitive state of patients for the clinician. *J. Psychiatr. Res.* 12(3), 189–198 (1975).

46 Lyness SA, Zarow C, Chui HC. Neuron loss in key cholinergic and aminergic nuclei in Alzheimer disease: a meta-analysis. *Neurobiol. Aging* 24(1), 1–23 (2003).

47 Higuchi M, Yanai K, Okamura N *et al.* Histamine H(1) receptors in patients with Alzheimer's disease assessed by positron emission tomography. *Neuroscience* 99(4), 721–729 (2000).

48 Ulas J, Brunner LC, Nguyen L, Cotman CW. Reduced density of adenosine A1 receptors and preserved coupling of adenosine A1 receptors to G proteins in Alzheimer hippocampus: a quantitative autoradiographic study. *Neuroscience* 52(4), 843–854 (1993).

49 Jucker M, Walker LC. Pathogenic protein seeding in Alzheimer disease and other neurodegenerative disorders. *Ann. Neurol.* 70(4), 532–540 (2011).

50 Spires-Jones TL, Stoothoff WH, De Calignon A, Jones PB, Hyman BT. Tau pathophysiology in neurodegeneration: a tangled issue. *Trends Neurosci.* 32(3), 150–159 (2009).

51 Iqbal K, Liu F, Gong CX, Alonso Adel C, Grundke-Iqbal I. Mechanisms of tau-induced neurodegeneration. *Acta Neuropathol.* 118(1), 53–69 (2009).

52 De Calignon A, Polydoro M, Suarez-Calvet M *et al.* Propagation of tau pathology in a model of early Alzheimer's disease. *Neuron* 73(4), 685–697 (2012).

53 Buerger K, Teipel SJ, Zinkowski R *et al.* CSF tau protein phosphorylated at threonine 231 correlates with cognitive decline in MCI subjects. *Neurology* 59(4), 627–629 (2002).

54 Augustinack JC, Schneider A, Mandelkow EM, Hyman BT. Specific tau phosphorylation sites correlate with severity of neuronal cytopathology in Alzheimer's disease. *Acta Neuropathol.* 103(1), 26–35 (2002).

55 Blennow K, Hampel H. CSF markers for incipient Alzheimer's disease. *Lancet Neurol.* 2(10), 605–613 (2003).

56 Xia CF, Arteaga J, Chen G *et al.* [^{18}F]T807, a novel tau positron emission tomography imaging agent for Alzheimer's disease. *Alzheimers Dement.* 9(6), 666–676 (2013).

57 Okamura N, Suemoto T, Furumoto S *et al.* Quinoline and benzimidazole derivatives: candidate probes for *in vivo* imaging of tau pathology in Alzheimer's disease. *J. Neurosci.* 25(47), 10857–10862 (2005).

58 Harada R, Okamura N, Furumoto S *et al.* Comparison of the binding characteristics of [^{18}F]THK-523 and other amyloid imaging tracers to Alzheimer's disease pathology. *Eur. J. Nucl. Med. Mol. Imaging* 40(1), 125–132 (2013).

59 Fodero-Tavoletti MT, Furumoto S, Taylor L *et al.* Assessing THK523 selectivity for tau deposits in Alzheimer's disease and non Alzheimer's disease tauopathies. *Alzheimers Res. Ther.* 6(1), 11 (2014).

60 Villemagne VL, Furumoto S, Fodero-Tavoletti MT *et al.* *In vivo* evaluation of a novel tau imaging tracer for Alzheimer's disease. *Eur. J. Nucl. Med. Mol. Imaging* 41(5), 816–826, (2014).

61 Sokoloff L, Reivich M, Kennedy C *et al.* The [^{14}C]deoxyglucose method for the measurement of local cerebral glucose utilization: theory, procedure, and normal values in the conscious and anesthetized albino rat. *J. Neurochem.* 28(5), 897–916 (1977).

62 Rocher AB, Chapon F, Blaizot X, Baron JC, Chavoix C. Resting-state brain glucose utilization as measured by PET is directly related to regional synaptophysin levels: a study in baboons. *Neuroimage* 20(3), 1894–1898 (2003).

63 Minoshima S, Giordani B, Berent S, Frey KA, Foster NL, Kuhl DE. Metabolic reduction in the posterior cingulate cortex in very early Alzheimer's disease. *Ann. Neurol.* 42(1), 85–94 (1997).

64 Mosconi L. Brain glucose metabolism in the early and specific diagnosis of Alzheimer's disease. FDG-PET studies in MCI and AD. *Eur. J. Nucl. Med. Mol. Imaging* 32(4), 486–510 (2005).

65 Silverman DH, Small GW, Phelps ME. Clinical value of neuroimaging in the diagnosis of dementia. Sensitivity and specificity of regional cerebral metabolic and other parameters for early identification of Alzheimer's Disease. *Clin. Positron Imaging* 2(3), 119–130 (1999).

66 Nestor PJ, Caine D, Fryer TD, Clarke J, Hodges JR. The topography of metabolic deficits in posterior cortical atrophy (the visual variant of Alzheimer's disease) with FDG-PET. *J. Neurol. Neurosurg. Psychiatry* 74(11), 1521–1529 (2003).

67 Rabinovici GD, Jagust WJ, Furst AJ *et al.* Abeta amyloid and glucose metabolism in three variants of primary progressive aphasia. *Ann. Neurol.* 64(4), 388–401 (2008).

* **Shows focal changes in metabolism, but no in patterns of amyloid deposition, across AD subtypes.**

68 Kim EJ, Cho SS, Jeong Y *et al.* Glucose metabolism in early onset versus late onset Alzheimer's disease: an SPM analysis of 120 patients. *Brain* 128(Pt 8), 1790–1801 (2005).

69 Jacobs D, Sano M, Marder K *et al.* Age at onset of Alzheimer's disease: relation to pattern of cognitive dysfunction and rate of decline. *Neurology* 44(7), 1215–1220 (1994).

70 Katzman R, Brown T, Thal LJ *et al.* Comparison of rate of annual change of mental status score in four independent studies of patients with Alzheimer's disease. *Ann. Neurol.* 24(3), 384–389 (1988).

71 Mosconi L, Brys M, Glodzik-Sobanska L, De Santi S, Rusinek H, De Leon MJ. Early detection of Alzheimer's disease using neuroimaging. *Exp. Gerontol.* 42(1–2), 129–138 (2007).

72 Nestor PJ, Fryer TD, Smielewski P, Hodges JR. Limbic hypometabolism in Alzheimer's disease and mild cognitive impairment. *Ann. Neurol.* 54(3), 343–351 (2003).

73 De Santi S, De Leon MJ, Rusinek H *et al.* Hippocampal formation glucose metabolism and volume losses in MCI and AD. *Neurobiol. Aging* 22(4), 529–539 (2001).

74 Mosconi L, De Santi S, Li Y *et al.* Visual rating of medial temporal lobe metabolism in mild cognitive impairment and Alzheimer's disease using FDG-PET. *Eur. J. Nucl. Med. Mol. Imaging* 33(2), 210–221 (2006).

75 Chetelat G, Desgranges B, De La Sayette V, Viader F, Eustache F, Baron JC. Mild cognitive impairment: Can FDG-PET predict who is to rapidly convert to Alzheimer's disease? *Neurology* 60(8), 1374–1377 (2003).

76 Mosconi L, Perani D, Sorbi S *et al.* MCI conversion to dementia and the APOE genotype: a prediction study with FDG-PET. *Neurology* 63(12), 2332–2340 (2004).

77 Drzezga A, Lautenschlager N, Siebner H *et al.* Cerebral metabolic changes accompanying conversion of mild cognitive impairment into Alzheimer's disease: a PET follow-up study. *Eur. J. Nucl. Med. Mol. Imaging* 30(8), 1104–1113 (2003).

78 Herholz K, Nordberg A, Salmon E *et al.* Impairment of neocortical metabolism predicts progression in Alzheimer's disease. *Dement. Geriatr. Cogn. Disord.* 10(6), 494–504 (1999).

79 Anchisi D, Borroni B, Franceschi M *et al.* Heterogeneity of brain glucose metabolism in mild cognitive impairment and clinical progression to Alzheimer disease. *Arch. Neurol.* 62(11), 1728–1733 (2005).

80 Herholz K, Salmon E, Perani D *et al.* Discrimination between Alzheimer dementia and controls by automated analysis of multicenter FDG PET. *Neuroimage* 17(1), 302–316 (2002).

81 Rossi S, Cappa SF, Babiloni C *et al.* Prefrontal [correction of Prefontal] cortex in long-term memory: an "interference" approach using magnetic stimulation. *Nat. Neurosci.* 4(9), 948–952 (2001).

82 Desgranges B, Baron JC, Eustache F. The functional neuroanatomy of episodic memory: the role of the frontal lobes, the hippocampal formation, and other areas. *Neuroimage* 8(2), 198–213 (1998).

83 De Leon MJ, Convit A, Wolf OT *et al.* Prediction of cognitive decline in normal elderly subjects with 2-[(^{18}F)fluoro-2-deoxy-D-glucose/poitron-emission tomography (FDG/PET). *Proc. Natl Acad. Sci. USA* 98(19), 10966–10971 (2001).

84 Mosconi L, De Santi S, Li J *et al.* Hippocampal hypometabolism predicts cognitive decline from normal aging. *Neurobiol. Aging* 29(5), 676–692 (2008).

85 Mosconi L, Mistur R, Switalski R *et al.* FDG-PET changes in brain glucose

metabolism from normal cognition to pathologically verified Alzheimer's disease. *Eur. J. Nucl. Med. Mol. Imaging* 36(5), 811–822 (2009).

86 Drzezga A, Grimmer T, Riemenschneider M *et al.* Prediction of individual clinical outcome in MCI by means of genetic assessment and (18)F-FDG PET. *J. Nucl. Med.* 46(10), 1625–1632 (2005).

87 Bateman RJ, Xiong C, Benzinger TL *et al.* Clinical and biomarker changes in dominantly inherited Alzheimer's disease. *N. Engl. J. Med.* 367(9), 795–804 (2012).

88 Mosconi L, Brys M, Switalski R *et al.* Maternal family history of Alzheimer's disease predisposes to reduced brain glucose metabolism. *Proc. Natl Acad. Sci. USA* 104(48), 19067–19072 (2007).

89 Knopman DS, Jack CR, Jr., Wiste HJ *et al.* Short-term clinical outcomes for stages of NIA-AA preclinical Alzheimer disease. *Neurology* 78(20), 1576–1582 (2012).

90 Knopman DS, Jack CR, Jr., Wiste HJ *et al.* Brain injury biomarkers are not dependent on beta-amyloid in normal elderly. *Ann. Neurol.* 73(4), 472–480 (2013).

** **Findings from the SNAP population suggesting that neurodegeneration in AD may not depend on the presence of β-amyloid.**

91 Mcgeer EG, Mcgeer PL. Inflammatory processes in Alzheimer's disease. *Prog. Neuropsychopharmacol. Biol. Psychiatry* 27(5), 741–749 (2003).

92 Vehmas AK, Kawas CH, Stewart WF, Troncoso JC. Immune reactive cells in senile plaques and cognitive decline in Alzheimer's disease. *Neurobiol. Aging* 24(2), 321–331 (2003).

93 Akiyama H, Barger S, Barnum S *et al.* Inflammation and Alzheimer's disease. *Neurobiol. Aging* 21(3), 383–421 (2000).

94 Groom GN, Junck L, Foster NL, Frey KA, Kuhl DE. PET of peripheral benzodiazepine binding sites in the microglionis of Alzheimer's disease. *J. Nucl. Med.* 36(12), 2207–2210 (1995).

95 Edison P, Archer HA, Gerhard A *et al.* Microglia, amyloid, and cognition in Alzheimer's disease: An [^{11}C](R)PK11195-PET and [^{11}C]PIB-PET study. *Neurobiol. Dis.* 32(3), 412–419 (2008).

96 Tomasi G, Edison P, Bertoldo A *et al.* Novel reference region model reveals increased microglial and reduced vascular binding of ^{11}C-(R)-PK11195 in patients with Alzheimer's disease. *J. Nucl. Med.* 49(8), 1249–1256 (2008).

97 Yasuno F, Ota M, Kosaka J *et al.* Increased binding of peripheral benzodiazepine receptor in Alzheimer's disease measured by positron emission tomography with [^{11}C]DAA1106. *Biol. Psychiatry* 64(10), 835–841 (2008).

98 Higuchi M. Visualization of brain amyloid and microglial activation in mouse models of Alzheimer's disease. *Curr. Alzheimer Res.* 6(2), 137–143 (2009).

99 Maeda J, Zhang MR, Okauchi T *et al.* *In vivo* positron emission tomographic imaging of glial responses to amyloid-beta and tau pathologies in mouse models of Alzheimer's disease and related disorders. *J. Neurosci.* 31(12), 4720–4730 (2011).

100 Wischik CM, Edwards PC, Lai RY, Roth M, Harrington CR. Selective inhibition of Alzheimer disease-like tau aggregation by phenothiazines. *Proc. Natl Acad. Sci. USA* 93(20), 11213–11218 (1996).

101 Varrone A, Mattsson P, Forsberg A *et al.* *In vivo* imaging of the 18-kDa translocator protein (TSPO) with [^{18}F]FEDAA1106 and PET does not show increased binding in Alzheimer's disease patients. *Eur. J. Nucl. Med. Mol. Imaging* 40(6), 921–931 (2013).

102 Miyoshi M, Ito H, Arakawa R *et al.* Quantitative analysis of peripheral benzodiazepine receptor in the human brain using PET with (^{11})C-AC-5216. *J. Nucl. Med.* 50(7), 1095–1101 (2009).

103 Horti AG, Gao Y, Ravert HT *et al.* Synthesis and biodistribution of [^{11}C]A-836339, a new potential radioligand for PET imaging of cannabinoid type 2 receptors (CB2). *Bioorg. Med. Chem.* 18(14), 5202–5207 (2010).

104 Verkhratsky A, Olabarria M, Noristani HN, Yeh CY, Rodriguez JJ. Astrocytes in Alzheimer's disease. *Neurotherapeutics* 7(4), 399–412 (2010).

105 Fowler JS, Macgregor RR, Wolf AP *et al.* Mapping human brain monoamine oxidase A and B with ^{11}C-labeled suicide inactivators and PET. *Science* 235(4787), 481–485 (1987).

106 Fowler JS, Volkow ND, Wang GJ *et al.* Age-related increases in brain monoamine oxidase B in living healthy human subjects. *Neurobiol. Aging* 18(4), 431–435 (1997).

107 Carter SF, Scholl M, Almkvist O *et al.* Evidence for astrocytosis in prodromal Alzheimer disease provided by ^{11}C-deuterium-L-deprenyl: a multitracer PET paradigm combining ^{11}C-Pittsburgh compound B and 18F-FDG. *J. Nucl. Med.* 53(1), 37–46 (2012).

* **Recent study with ^{11}C-deuterium-L-deprenyl suggesting that astrocytosis occurs in prodromal AD.**

108 Sun GY, Horrocks LA, Farooqui AA. The roles of NADPH oxidase and phospholipases A2 in oxidative and inflammatory responses in neurodegenerative diseases. *J. Neurochem.* 103(1), 1–16 (2007).

109 Esposito G, Giovacchini G, Liow JS *et al.* Imaging neuroinflammation in Alzheimer's disease with radiolabeled arachidonic acid and PET. *J. Nucl. Med.* 49(9), 1414–1421 (2008).

110 Dennis EA. Diversity of group types, regulation, and function of phospholipase A2. *J. Biol. Chem.* 269(18), 13057–13060 (1994).

111 Weichel O, Hilgert M, Chatterjee SS, Lehr M, Klein J. Bilobalide, a constituent of Ginkgo biloba, inhibits NMDA-induced phospholipase A2 activation and phospholipid breakdown in rat hippocampus. *Naunyn. Schmiedebergs Arch. Pharmacol.* 360(6), 609–615 (1999).

112 Bazelin M, Chang L, Bell JM, Rapoport SI. Chronic lithium chloride administration attenuates brain NMDA receptor-initiated signaling via arachidonic acid in unanesthetized rats. *Neuropsychopharmacology* 31(8), 1659–1674 (2006).

113 Mcgeer PL, Mcgeer EG. Glial cell reactions in neurodegenerative diseases: pathophysiology and therapeutic interventions. *Alzheimer Dis. Assoc. Disord.* 12(Suppl. 2), S1–S6 (1998).

114 Montine KS, Quinn JF, Zhang J *et al.* Isoprostanes and related products of lipid peroxidation in neurodegenerative diseases. *Chem. Phys. Lipids* 128(1–2), 117–124 (2004).

115 Greenamyre JT, Maragos WF, Albin RL, Penney JB, Young AB. Glutamate transmission and toxicity in Alzheimer's disease. *Prog. Neuropsychopharmacol. Biol. Psychiatry* 12(4), 421–430 (1988).

116 Davis KL, Mohs RC, Marin D *et al.* Cholinergic markers in elderly patients with early signs of Alzheimer disease. *JAMA* 281(15), 1401–1406 (1999).

117 Perry EK, Perry RH, Blessed G, Tomlinson BE. Necropsy evidence of central cholinergic deficits in senile dementia. *Lancet* 1(8004), 189 (1977).

118 Francis PT, Palmer AM, Snape M, Wilcock GK. The cholinergic hypothesis of Alzheimer's disease: a review of progress. *J. Neurol. Neurosurg. Psychiatry* 66(2), 137–147 (1999).

119 Kilbourn MR, Snyder SE, Sherman PS, Kuhl DE. *In vivo* studies of acetyl-cholinesterase activity using a labeled substrate, N-[^{11}C]methylpiperdin-4-yl propionate ([^{11}C]PMP). *Synapse* 22(2), 123–131 (1996).

120 Namba H, Iric T, Fukushi K, Iyo M. *In vivo* measurement of acetylcholinesterase activity in the brain with a radioactive acetylcholine analog. *Brain Res.* 667(2), 278–282 (1994).

121 Kuhl DE, Koeppe RA, Minoshima S *et al.* *In vivo* mapping of cerebral acetylcholinesterase activity in aging and Alzheimer's disease. *Neurology* 52(4), 691–699 (1999).

122 Iyo M, Namba H, Fukushi K *et al.* Measurement of acetylcholinesterase by positron emission tomography in the brains of healthy controls and patients with Alzheimer's disease. *Lancet* 349(9068), 1805–1809 (1997).

123 Herholz K, Bauer B, Wienhard K *et al.* *In-vivo* measurements of regional acetylcholine esterase activity in degenerative dementia: comparison with blood flow and glucose metabolism. *J. Neural Transm.* 107(12), 1457–1468 (2000).

124 Rinne JO, Kaasinen V, Jarvenpaa T *et al.* Brain acetylcholinesterase activity in mild cognitive impairment and early Alzheimer's disease. *J. Neurol. Neurosurg. Psychiatry* 74(1), 113–115 (2003).

125 Kaasinen V, Nagren K, Jarvenpaa T *et al.* Regional effects of donepezil and rivastigmine on cortical acetylcholinesterase activity in Alzheimer's disease. *J. Clin. Pharmacol.* 22(6), 615–620 (2002).

126 Kadir A, Darreh-Shori T, Almkvist O *et al.* PET imaging of the *in vivo* brain acetylcholinesterase activity and nicotine binding in galantamine-treated patients with AD. *Neurobiol. Aging* 29(8), 1204–1217 (2008).

127 Zubieta JK, Koeppe RA, Frey KA *et al.* Assessment of muscarinic receptor concentrations in aging and Alzheimer disease with [^{11}C]NMPB and PET. *Synapse* 39(4), 275–287 (2001).

128 Cohen RM, Podruchny TA, Bokde AL *et al.* Higher *in vivo* muscarinic-2 receptor distribution volumes in aging subjects with an apolipoprotein E-epsilon4 allele. *Synapse* 49(3), 150–156 (2003).

129 Sihver W, Langstrom B, Nordberg A. Ligands for *in vivo* imaging of nicotinic receptor subtypes in Alzheimer brain. *Acta Neurol. Scand.* 176 (Suppl.), 27–33 (2000).

130 Kadir A, Almkvist O, Wall A, Langstrom B, Nordberg A. PET imaging of cortical ^{11}C-nicotine binding correlates with the cognitive function of attention in Alzheimer's disease. *Psychopharmacology (Berlin)* 188(4), 509–520 (2006).

131 Kadir A, Darreh-Shori T, Almkvist O, Wall A, Langstrom B, Nordberg A. Changes in brain ^{11}C-nicotine binding sites in patients with mild Alzheimer's disease following rivastigmine treatment as assessed by PET. *Psychopharmacology (Berlin)* 191(4), 1005–1014 (2007).

132 Ellis JR, Villemagne VL, Nathan PJ *et al.* Relationship between nicotinic receptors and cognitive function in early Alzheimer's disease: a 2-[^{18}F]fluoro-A-85380 PET study. *Neurobiol. Learn. Mem.* 90(2), 404–412 (2008).

133 Brust P, Patt JT, Deuther-Conrad W *et al.* *In vivo* measurement of nicotinic acetylcholine receptors with [^{18}F]norchloro-fluoro-homoepibatidine. *Synapse* 62(3), 205–218 (2008).

134 Kem WR. The brain alpha7 nicotinic receptor may be an important therapeutic target for the treatment of Alzheimer's disease: studies with DMXBA (GTS-21). *Behav. Brain Res.* 113(1–2), 169–181 (2000).

135 Pomper MG, Phillips E, Fan H *et al.* Synthesis and biodistribution of radiolabeled alpha 7 nicotinic acetylcholine receptor ligands. *J. Nucl. Med.* 46(2), 326–334 (2005).

136 Tyrrell PJ, Sawle GV, Ibanez V *et al.* Clinical and positron emission tomographic studies in the 'extrapyramidal syndrome' of dementia of the Alzheimer type. *Arch. Neurol.* 47(12), 1318–1323 (1990).

137 Kemppainen N, Ruottinen H, Nagren K, Rinne JO. PET shows that striatal dopamine D1 and D2 receptors are differentially affected in AD. *Neurology* 55(2), 205–209 (2000).

138 Tanaka Y, Meguro K, Yamaguchi S *et al.* Decreased striatal D2 receptor density associated with severe behavioral abnormality in Alzheimer's disease. *Ann. Nucl. Med.* 17(7), 567–573 (2003).

139 Kemppainen N, Laine M, Laakso MP *et al.* Hippocampal dopamine D2 receptors correlate with memory functions in Alzheimer's disease. *Eur. J. Neurosci.* 18(1), 149–154 (2003).

140 Rinne JO, Sahlberg N, Ruottinen H, Nagren K, Lehikoinen P. Striatal uptake of the dopamine reuptake ligand [^{11}C]beta-CFT is reduced in Alzheimer's disease assessed by positron emission tomography. *Neurology* 50(1), 152–156 (1998).

141 Koeppe RA, Gilman S, Joshi A *et al.* ^{11}C-DTBZ and ^{18}F-FDG PET measures in differentiating dementias. *J. Nucl. Med.* 46(6), 936–944 (2005).

142 Koeppe RA, Gilman S, Junck L, Wernette K, Frey KA. Differentiating Alzheimer's disease from dementia with Lewy bodies and Parkinson's disease with (+)-[^{11}C] dihydrotetrabenazine positron emission tomography. *Alzheimers Dement.* 4(1 Suppl. 1), S67–S76 (2008).

143 Gilman S, Koeppe RA, Little R *et al.* Striatal monoamine terminals in Lewy body dementia and Alzheimer's disease. *Ann. Neurol.* 55(6), 774–780 (2004).

144 Lanctot KL, Herrmann N, Mazzotta P. Role of serotonin in the behavioral and psychological symptoms of dementia. *J. Neuropsychiatry Clin. Neurosci.* 13(1), 5–21 (2001).

145 Mann DM, Yates PO. Serotonin nerve cells in Alzheimer's disease. *J. Neurol. Neurosurg. Psychiatry* 46(1), 96 (1983).

146 Palmer AM, Francis PT, Benton JS *et al.* Presynaptic serotonergic dysfunction in patients with Alzheimer's disease. *J. Neurochem.* 48(1), 8–15 (1987).

147 Cross AJ, Crow TJ, Johnson JA *et al.* Studies on neurotransmitter receptor systems in neocortex and hippocampus in senile dementia of the Alzheimer-type. *J. Neurol. Sci.* 64(2), 109–117 (1984).

148 Palacios JM, Waeber C, Hoyer D, Mengod G. Distribution of serotonin receptors. *Ann. N. Y. Acad. Sci.* 600, 36–52 (1990).

149 Chen CP, Eastwood SL, Hope T, Mcdonald B, Francis PT, Esiri MM. Immunocytochemical study of the dorsal and median raphe nuclei in patients with Alzheimer's disease prospectively assessed for behavioural changes. *Neuropathol. Appl. Neurobiol.* 26(4), 347–355 (2000).

150 Yamamoto T, Hirano A. Nucleus raphe dorsalis in Alzheimer's disease: neurofibrillary tangles and loss of large neurons. *Ann. Neurol.* 17(6), 573–577 (1985).

151 Zubenko GS, Moossy J, Martinez AJ *et al.* Neuropathologic and neurochemical correlates of psychosis in primary dementia. *Arch. Neurol.* 48(6), 619–624 (1991).

152 Ouchi Y, Yoshikawa E, Sekine Y *et al.* Microglial activation and dopamine terminal loss in early Parkinson's disease. *Ann. Neurol.* 57(2), 168–175 (2005).

153 Blin J, Baron JC, Dubois B *et al.* Loss of brain 5-HT2 receptors in Alzheimer's disease. *In vivo* assessment with positron emission tomography and [18F]setoperone. *Brain* 116 (Pt 3), 497–510 (1993).

154 Meltzer CC, Smith G, Price JC *et al.* Reduced binding of [^{18}F]altanserin to serotonin type 2A receptors in aging: persistence of effect after partial volume correction. *Brain Res.* 813(1), 167–171 (1998).

155 Kepe V, Barrio JR, Huang SC *et al.* Serotonin 1A receptors in the living brain of Alzheimer's disease patients. *Proc. Natl Acad. Sci. USA* 103(3), 702–707 (2006).

156 Madsen K, Neumann W-J, Holst K *et al.* Cerebral serotonin 4 receptors and amyloid-beta in early Alzheimer's Disease. *J. Alzheimers Dis.* 26(3), 457–466 (2011).

157 Tejani-Butt SM, Yang J, Zaffar H. Norepinephrine transporter sites are decreased in the locus coeruleus in Alzheimer's disease. *Brain Res.* 631(1), 147–150 (1993).

158 Zarow C, Lyness SA, Mortimer JA, Chui HC. Neuronal loss is greater in the locus coeruleus than nucleus basalis and substantia nigra in Alzheimer and Parkinson diseases. *Arch. Neurol.* 60(3), 337–341 (2003).

159 Gulyas B, Brockschnieder D, Nag S *et al.* The norepinephrine transporter (NET) radioligand (*S,S*)-[^{18}F]FMeNER-D2 shows significant decreases in NET density in the human brain in Alzheimer's disease: a post-mortem autoradiographic study. *Neurochem. Int.* 56(6–7), 789–798 (2010).

160 Naddafi F, Mirshafiey A. The neglected role of histamine in Alzheimer's disease. *Am. J. Alzheimers Dis. Other Demen.* 28(4), 327–336 (2013).

161 Tiligada E, Kyriakidis K, Chazot PL, Passani MB. Histamine pharmacology and new CNS drug targets. *CNS Neurosci. Ther.* 17(6), 620–628 (2011).

162 Lintunen M, Sallmen T, Karlstedt K, Panula P. Transient changes in the limbic histaminergic system after systemic kainic acid-induced seizures. *Neurobiol. Dis.* 20(1), 155–169 (2005).

163 Knob GF, Bloom FE. Behavioral effects of neuropeptides: endorphins and vasopressin. *Annu. Rev. Physiol.* 44, 571–582 (1982).

164 Cohen MR, Cohen RM, Pickar D, Weingartner H, Murphy DL, Bunney WE Jr. Behavioural effects after high dose naloxone administration to normal volunteers. *Lancet* 2(8255), 1110 (1981).

165 Cohen MR, Cohen RM, Pickar D, Weingartner H, Murphy DL. High-dose naloxone infusions in normals. Dose-dependent behavioral, hormonal, and physiological responses. *Arch. Gen. Psychiatry* 40(6), 613–619 (1983).

166 Barg J, Belcheva M, Rowinski J *et al.* Opioid receptor density changes in Alzheimer amygdala and putamen. *Brain Res.* 632(1–2), 209–215 (1993).

167 Hiller JM, Itzhak Y, Simon EJ. Selective changes in mu, delta and kappa opioid receptor binding in certain limbic regions of the brain in Alzheimer's disease patients. *Brain Res.* 406(1–2), 17–23 (1987).

168 Cohen R, Andreason P, Doudet D, Carson R, Sunderland T. Opiate receptor avidity and cerebral blood flow in Alzheimer's Disease. *J. Neurol. Sci.* 148(2), 171–180 (1997).

169 Jansen KL, Faull RL, Dragunow M, Synek BL. Alzheimer's disease: changes in hippocampal *N*-methyl-D-aspartate, quisqualate, neurotensin, adenosine, benzodiazepine, serotonin and opioid receptors-an autoradiographic study. *Neuroscience* 39(3), 613–627 (1990).

170 Jaarsma D, Sebens JB, Korf J. Reduction of adenosine A1-receptors in the perforant pathway terminal zone in Alzheimer hippocampus. *Neurosci. Lett.* 121(1–2), 111–114 (1991).

171 Kalaria RN, Sromek S, Wilcox BJ, Unnerstall JR. Hippocampal adenosine A1 receptors are decreased in Alzheimer's disease. *Neurosci. Lett.* 118(2), 257–260 (1990).

172 Meguro K, Lemestric C, Landeau B, Desgranges B, Eustache F, Baron JC. Relations between hypometabolism in the posterior association neocortex and hippocampal atrophy in Alzheimer's disease: a PET/MRI correlative study. *J. Neurol. Neurosurg. Psychiatry* 71(3), 315–321 (2001).

173 Ishii K, Sasaki H, Kono AK, Miyamoto N, Fukuda T, Mori E. Comparison of gray matter and metabolic reduction in mild Alzheimer's disease using FDG-PET and voxel-based morphometric MR studies. *Eur. J. Nucl. Med. Mol. Imaging* 32(8), 959–963 (2005).

174 Fukumitsu N, Ishii K, Kimura Y *et al.* Adenosine A(1) receptors using 8-dicyclopropylmethyl-1-[(11)C]methyl-3-propylxanthine PET in Alzheimer's disease. *Ann. Nucl. Med.* 22(10), 841–847 (2008).

175 Dragunow M, Murphy K, Leslie RA, Robertson HA. Localization of adenosine A1-receptors to the terminals of the perforant path. *Brain Res.* 462(2), 252–257 (1988).

176 Owen DR, Yeo AJ, Gunn RN *et al.* An 18-kDa translocator protein (TSPO) polymorphism explains differences in binding affinity of the PET radioligand PBR28. *J. Cereb. Blood Flow Metab.* 32(1), 1–5 (2012).

177 Mizrahi R, Rusjan PM, Kennedy J *et al.* Translocator protein (18 kDa) polymorphism (rs6971) explains in-vivo brain binding affinity of the PET radioligand [(18)F]-FEPPA. *J. Cereb. Blood Flow Metab.* 32(6), 968–972 (2012).

178 Garraux G, Salmon E. Clinical contribution of PET neurotransmission imaging in neurological disorders. *Acta Neurol. Belg.* 105(3), 119–136 (2005).

179 Kadir A, Nordberg A. Target-specific PET probes for neurodegenerative disorders related to dementia. *J. Nucl. Med.* 51(9), 1418–1430 (2010).

180 Herholz K, Carter SF, Jones M. Positron emission tomography imaging in dementia. *Br. J. Radiol.* 80(Spec. No. 2), S160–S167 (2007).

Discussão Artigo 2

No artigo “Nonamyloid PET biomarkers and Alzheimer’s disease: current and future perspectives” publicado no periódico Future Neurology em 2014 o objetivo foi realizar uma ampla revisão sobre a neuroimagem através do exame de PET na DA sob uma abordagem não relacionada com os radiotraçadores para amiloide.

Na fisiopatologia da DA observa-se, além do acúmulo de proteínas patogênicas, alterações neuroinflamatórias e neurodegenerativas; bem como a disfunção de diversos sistemas de neurotransmissores. A análise através do PET oferece a possibilidade de avaliar diferentes processos cerebrais relacionados com a DA *in vivo* e de forma dinâmica.

A imagem molecular, através de uma ampla gama de radiotraçadores com diferentes características, permite a correlação entre diferentes aspectos clínicos e o desenvolvimento de processos patológicos na DA e oferece melhores perspectivas nas estratégias de prevenção, investigação e desenvolvimento de terapias mais efetivas para a doença.

ANEXO C - Artigo 3: Imaging Alzheimer's disease pathophysiology with PET.

Dement Neuropsychol 2016 June;10(2):79-90

Imaging Alzheimer's disease pathophysiology with PET

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ABSTRACT. Alzheimer's disease (AD) has been reconceptualised as a dynamic pathophysiological process characterized by preclinical, mild cognitive impairment (MCI), and dementia stages. Positron emission tomography (PET) associated with various molecular imaging agents reveals numerous aspects of dementia pathophysiology, such as brain amyloidosis, tau accumulation, neuroreceptor changes, metabolism abnormalities and neuroinflammation in dementia patients. In the context of a growing shift toward presymptomatic early diagnosis and disease-modifying interventions, PET molecular imaging agents provide an unprecedented means of quantifying the AD pathophysiological process, monitoring disease progression, ascertaining whether therapies engage their respective brain molecular targets, as well as quantifying pharmacological responses. In the present study, we highlight the most important contributions of PET in describing brain molecular abnormalities in AD.

Key words: Alzheimer's disease, positron emission tomography, amyloid imaging, neuroinflammation, neurodegeneration, tau.

A FISIOPATOLOGIA DA DOENÇA DE ALZHEIMER ATRAVÉS DO PET

RESUMO. A doença de Alzheimer tem sido reconceitualizada como um processo patofisiológico dinâmico caracterizado pelos estágios pré-clínico, comprometimento cognitivo leve e demência. A tomografia por emissão de pósitrons associada a vários agentes de imagem molecular revela numerosos aspectos da patofisiologia da demência tais como amiloidose cerebral, acúmulo de tau, mudanças em neurorreceptores, anormalidades de metabolismo e neuroinflamação nestes pacientes. No contexto de um crescimento em direção ao diagnóstico precoce pré-sintomático e intervenções modificadores da doença, a imagem de PET com agentes moleculares fornece meio para quantificar o processo patofisiológico da DA sem precedentes, monitorizar a progressão da doença, bem como quantificar resposta farmacológica. Aqui, nós realçamos as mais importantes contribuições do PET na descrição de anormalidades moleculares cerebrais na DA.

Palavras-chave: doença de Alzheimer, tomografia por emissão de positron, imagem amilóide, neuroinflamação, neurodegeneração, tau.

INTRODUCTION

Alzheimer's disease (AD) was first described in 1906, when the German psychiatrist Alois Alzheimer reported the clinical and pathological features from a patient called Auguste Deter. At age 51, she became afflicted by an obscure progressive neuropsychiatric condition characterized by severe cognitive decline associated with behavioral symptoms. Five years after admission, she became mute and confined to her bed. Similarly to many patients, her death came as a consequence of septicemia. At the necropsy, the brain histopathological examination revealed the presence of amyloid plaques and neurofibrillary tangles, both of which later became known

This study was conducted at the Douglas Mental Health Research Institute, Montreal, Canada.

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Disclosure: The authors report no conflicts of interest.

Received December 10, 2015. Accepted in final form March 16, 2016.

■ Dement Neuropsychol 2016 June;10(2):79-90

as the neuropathological hallmarks of AD. The term "Alzheimer's disease" was introduced by the psychiatrist Emil Kraepelin in 1910, in his Handbook of Psychiatry.[1,2] Initially considered a rare disease, AD became recognized as a frequent condition in aging individuals as well as the leading cause of dementia.[3]

The neuropathological features of AD constitute the extracellular deposition of amyloid-β (Aβ) aggregates (senile plaques), intracellular inclusions of hyperphosphorylated tau aggregates (neurofibrillary tangles; NFTs), brain atrophy and cell depletion.[4] These features silently accumulate and propagate across brain regions for many years, leading to subsequent clinical and functional decline. At the stage of clinical symptoms of dementia, these pathophysiological processes have already significantly compromised a large proportion of brain circuits involved in cognition. In its typical presentation, AD is characterized by progressive cognitive impairment initially confined to the episodic memory system.

The research criteria for the diagnosis of AD were first defined in 1984 by a working group jointly established by the National Institute of Neurological and Communicative Disorders and Stroke (NINCDS) and the Alzheimer's Disease and Related Disorders Association (ADRDA).[5] The NINCDS-ADRDA criteria assumed a static association between the pathological and clinical characteristics of AD.[6] These criteria were useful and widely adopted, remaining in use for over 25 years. During the last decade, important advances in genetics, biochemistry, clinical, and pathological characterization of dementias have taken place, including the development of *in vivo* biomarkers of AD pathophysiology, leading to changes in the criteria for AD.[6] An International Working Group (IWG) initiated these revisions,[7,8] followed by the National Institute on Aging and Alzheimer's Association workgroup (NIA-AA), leading to new diagnostic criteria including predementia stages of AD. In particular, the NIA-AA research criteria encompasses asymptomatic "preclinical" AD,[9] mild cognitive impairment (MCI) due to AD,[10] and AD dementia stages.[11] This disease framework incorporates important advances such as the notion of clinical and pathophysiological progression, the genetic form of AD, as well as atypical presentations of AD. Importantly, AD pathophysiology now assumes a progressive cascade of events associated with Aβ toxicity, which triggers a series of downstream biochemical cascades including tau hyperphosphorylation, synaptic depletion,[12,13] neuroinflammation,[14] and abnormal neurotransmission.[15]

Positron emission tomography (PET) is a non-invasive method capable of quantifying biological processes based on the dynamic distribution of radiotracers injected during the scanning session. There are numerous PET molecular imaging agents, each of which is specifically designed to quantify a single molecular target. They allow for the characterization of abnormal protein aggregation (fibrillary Aβ or hyperphosphorylated tau deposits), metabolic abnormalities (glucose metabolism and cerebral blood flow), and neuroinflammation (astrocytosis, microgliosis and phospholipase activity). This review article focuses on PET molecular imaging in AD, reviewing the role of imaging biomarkers in the diagnosis and monitoring of key pathophysiological events of AD, which include Aβ and tau deposition, neurodegeneration, and neuroinflammation.

PET BIOMARKERS FOR AMYLOID DEPOSITION

Though the pathogenesis of AD remains unclear, the hallmark of this neurodegenerative disease is the deposition of Aβ plaques, together with other features, such as the presence of NFTs. Aβ deposits are known to progressively accumulate in certain brain regions over the course of the disease, beginning long before the clinical onset. The canonical PET molecular agent capable of detecting fibrillary Aβ *in vivo* is the carbon-11 labelled thioflavin T derivative 2-(4'-methylaminophenyl)-6-hydroxybenzothiazole, also known as [^{11}C]Pittsburgh Compound-B ([^{11}C]PiB). The most widely studied amyloid PET tracer, [^{11}C]PiB is considered the benchmark for PET-amyloid imaging.[16-18] The short half-life of carbon-11 (20 minutes), however, limits its use to centers possessing an on-site cyclotron and specialized radiochemistry. The new generation of amyloid ligands, labeled with fluorine-18, have a longer half-life of approximately 110 minutes. Due to these differences in half-life, these fluorine-18 labeled compounds can be regularly produced at a cyclotron site and distributed to other facilities (Table 1).[19,20]

Previous studies indicate that patients with MCI present 20-30% higher prevalence of amyloid positivity when compared to controls, which suggests that both amnestic and nonamnestic MCI are associated with an increased risk for AD. This association is much more relevant in the amnestic MCI subtype, but it is important to highlight that a large number of MCI patients are amyloid negative, supporting the theory that MCI is not always due to amyloid-related AD pathology.[21-25] A positive amyloid-PET scan increases the probability of conversion to AD;[21,26,27] however, the interval over which MCI amyloid positive patients may convert to AD dementia is variable, ranging from 1 to 5 years.[21,26] It is important to keep in mind the present limited clinical utility of detection of

Table 1. Summary of amyloid imaging agents currently available for quantifying brain amyloid.

	[^{11}C]PIB	[^{18}F]Flutemetamol	[^{18}F] Florbetapir	[^{18}F]Florbetaben	[^{18}F]NAV4694
	Research Use	Vizamyl®	Amivid®	Neuraceq®	Phase 3
Alternative name	–	GE-067	–	BAY-94-9172, AV-1	[^{18}F]AZD4694
Parent molecule	Benzothiazole	Benzothiazole	Styrylpyridine	Stilbene	Benzothiazole
Amyloid affinity (Ki, nM)	0.9	0.7	2.2.	2.4	0.7
Plasma metabolites	Polar	Polar	Polar and non-polar	Polar and non-polar	Polar
Typical injected dose (MBq)	250–450	250–450	300	300	300
Typical imaging time (min)	40–90	90–110	50–70	90–130	50-60

AD pathophysiology in MCI patients, given the lack of efficacious therapy as well as the ethical limitations when proposing amyloid imaging in patients meeting clinical criteria for MCI. On the other hand, positive amyloid-PET scans in MCI patients provides confirmation that this clinical situation occurs due to AD pathophysiology, supporting the idea of investing in non-pharmacological interventions, such as cognitive enrichment and diet and lifestyle changes. By contrast, a negative amyloid-PET scan in patients presenting cognitive impairment suggests other non-AD pathologies, such as fronto-temporal lobar degeneration, hippocampal sclerosis, or argyrophilic grain disease. Although Parkinson's disease (PD) and dementia with Lewy bodies (DLB) are neuro-degenerative disorders associated with brain deposition of fibrillary aggregates of α-synuclein protein, individuals with these synucleinopathies and cognitive decline have shown variability with regard to the presence of an abnormal amyloid-PET scan.[28,29] Individuals presenting with Parkinson's disease (PD) dementia have shown lower brain amyloid burden than AD, dementia with Lewy bodies, exhibiting similar burden to controls. On the other hand, patients presenting dementia with DLB have shown higher amyloid burden than controls, comparable to levels seen in AD patients.[20,21] Interestingly, these studies have suggested that a positive amyloid-PET scan in DLB individuals might be associated with more rapid clinical progression.[28,20]

The clinical importance of amyloid imaging has been extensively debated. In order to delineate scenarios in which the use of amyloid PET radiotracers is appropriate in the evaluation of cognitive impairment, the Alzheimer's Association and the Society of Nuclear Medicine and Molecular Imaging have set up an Amyloid Imaging Taskforce (AIT).[22] Performing a literature review in conjunction with expert opinion, the AIT defined a set of Appropriate Use Criteria (AUC) for clinical amyloid imaging, recommending that the use of amyloid imaging be limited to patients showing progressive and unexplained cognitive decline with uncertain diagnosis, early onset dementia and/or atypical clinical presentation, and when knowledge of amyloid status is expected to alter the therapeutic approach. Clinical utilities of amyloid imaging are restricted to specific cases. Patients with early-onset dementia (commonly defined as onset before 65 years of age) have a lower probability of AD pathophysiology underlying their cognitive decline than late-onset cases. In this regard, amyloid imaging might clarify whether underlying brain amyloidosis is associated with clinical syndromes such as primary progressive aphasia, dementias characterized by a predominance of executive dysfunction, visuospatial symptoms, progressive apraxia, and corticobasal syndrome. Given the economic and family impact of the diagnosis of AD in this population, an elevated level of diagnostic certainty is highly desirable. Furthermore, the presence of amyloid pathology might provide the rationale for proposing the clinical use of cholinesterase inhibitors in this population. The AIT contraindicates the use of amyloid imaging in asymptomatic people or in those with a cognitive complaint but no clinical confirmation of impairment, for determining the severity of dementia and for non-medical reasons, such as insurance, legal or employment decisions.

Finally, recently incorporated into AD research diagnostic criteria, amyloid PET plays a major role in defining AD. The IWG criteria classified amyloid PET as a useful biomarker which supports clinical diagnosis, especially when the presentation is atypical.[33] Hence, amyloid PET has increasingly been used in clinical trials, particularly for enriching study populations comprising individuals with a high probability of presenting AD or for monitoring target engagement of anti-amyloid therapies. How-

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ever, the clinical utility of amyloid PET has been increasingly discussed, particularly in the absence of effective disease modifying agents.[22,24,25] The ensuing paragraphs summarize progress regarding amyloid imaging in AD.

[^{11}C]Pittsburgh Compound-B. [^{11}C]PIB exhibits a high affinity and specificity for Aβ plaques, as opposed to Lewy bodies or tau proteins.[26,27] showing high accuracy in the evaluation of Aβ plaque burden. In individuals with AD, [^{11}C]PIB uptake is distributed in the frontal, medial and lateral posterior parietal cortices, precuneus, occipital cortex and lateral temporal cortices, as well as in the striatum (Figure 1).[16,28-40] Low [^{11}C]PIB uptake is typically observed in the cerebellar cortex.

A recent Cochrane review study on [^{11}C]PIB for early diagnosis of AD in individuals with MCI[41] estimated sensitivity of 96% (95% confidence interval: 87-99) and median specificity of 58%. A growing consensus emerging from longitudinal studies indicates that disease-modifying therapies targeting amyloid should be administered at very early stages of the disease.[42] Concerted efforts worldwide are focusing on advancing the diagnosis of AD to a preclinical stage.[43,44] Elevated [^{11}C]PIB binding in nondemented subjects indicates that it may be sensitive for the detection of preclinical AD,[45] suggesting a 20 to 30-year interval between first amyloid positivity and onset of dementia.[25]

Presently, [^{11}C]PIB is one of the most accurate agents for localizing and quantifying Aβ deposition; however, the short half-life of carbon-11 restricts its use to facilities with an onsite cyclotron and expertise in carbon-11 radiochemistry. Hence, the overall production and utilization costs are not generally affordable. In order to solve this issue, recent studies haves focused on fluorine-18 labeled radiopharmaceuticals, whose longer half-life allows for regional distribution and commercialization. Widely used as a biomarker in the diagnostic criteria for AD, cerebrospinal fluid (CSF) also confirms Aβ pathology, with CSF $A\beta_{1-42}$ having an inverse correlation with [^{11}C]PIB SUVR values.[46] However, one limitation of CSF $A\beta_{1-42}$ is that it does not provide any regionalized information on amyloid burden in the brain.

Fluorine-18 labeled ligands. While the short half-life of carbon labeled radiotracers limits the application of [^{11}C]PIB in clinical practice, fluorine-18 labeled amyloid PET radiopharmaceuticals have been developed, with a growing potential for clinical and research purposes. These compounds provide a new window of opportunity in the assessment of preclinical and clinical AD due to the 110-minute half-life of fluorine-18, a massive difference in terms of distribution logistics when compared to [^{11}C]PIB production. At present, four radiofluorinated labeled radiopharmaceuticals are drawing scientific attention: [^{18}F]3-F-PIB ([^{18}F]flutemetamol),[47] [^{18}F]AV- 45 ([^{18}F]florbetaptir),[48] [^{18}F]AV-1 or [^{18}F]BAY94–9172 ([^{18}F]florbetaben),[49,50] and [^{18}F]AZD4694 or [^{18}F]NAV4694.[51]

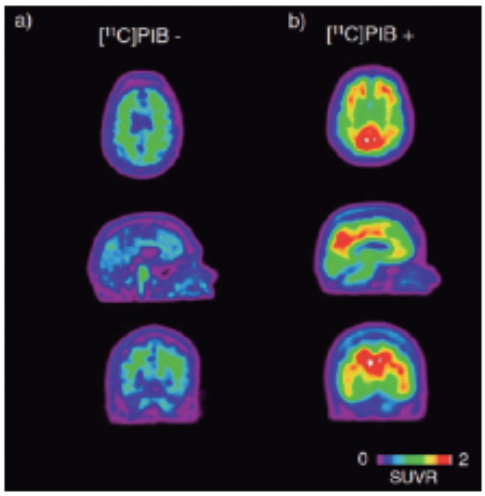

Figure 1. Amyloid signature. Representative [^{11}C]PIB PET images showing white matter uptake of [^{11}C]PIB in a patient with CBS (a; age 74, MMSE 23), and extensive cortical uptake in a patient with AD (b; age 70, MMSE 28).

Similarly to [^{11}C]PIB, these fluorine-18 amyloid tracers exhibit significant binding to fibrillar Aβ in the brain,[47,48] albeit with some differences. [^{18}F]Flutemetamol, [^{18}F]florbetaptir, and [^{18}F]florbetaben are less specific than [^{11}C]PIB, displaying white matter binding whereas [^{18}F]NAV4694 shows more specific grey-white matter demarcation and better pharmacokinetic properties as a potential competitor of [^{11}C]PIB PET. The Food and Drug Administration (FDA) and European Medicines Agency (EMA) have already approved [^{18}F]florbetaptir (Amyvid™), [^{18}F]flutemetamol (Vizamyl™), and [^{18}F]florbetaben (Neuraceq™) for use in clinical practice, while [^{18}F]NAV4694 is currently in phase III trials.[50,51]

While holding great research potential, all four compounds discriminate healthy controls from AD subjects with high accuracy. [^{18}F]Florbetaptir has shown remarkable accuracy in amyloid detection, with clinical application even in preclinical AD.[52,53] The diagnostic value of [^{18}F]flutemetamol has been tested recently in symptom-

atic and preclinical AD, showing relevance in detecting primarily advanced stages of Aβ deposition at both clinical and preclinical stages.[54] [18F]Florbetaben is a highly accurate Aβ PET tracer that has the potential to support the clinical diagnosis of AD and other causes of cognitive decline,[49] with similar visual and quantitative assessment in PET.

A third generation probe, [18F]AZD4694, called NAV4694, has been attracting growing attention for its near-identical imaging characteristics , but longer half-life to those of [11C]PIB. [18F]NAV4694 binds specifically to Aβ plaques, with excellent frontal cortex-to-white matter ratios both in AD and healthy controls.[51] The accuracy of [18F]NAV4694 in binding Aβ plaques has been tested, reliably discriminating AD patients from healthy controls, and satisfies requirements for clinical usage and evaluation of disease-modifying strategies in AD.[55] In a recent study, [18F]NAV4694 exhibited a significant overlap in amyloid imaging with [11C]PIB in patients with FTLD, theoretically an Aβ-free disease in most of its pathological subtypes.[56] Also, the linear correlation of [18F]NAV4694 with [11C]PIB of 0.95 is higher than the values reported for [18F]florbetapir (range 0.33-0.64) and [18F]florbetaben (0.71).[51]

PET BIOMARKERS FOR NEUROINFLAMMATION

A factor known to be involved in the pathogenesis of AD is the immune response, with initial association between amyloid deposits and immune response emerging among elderly in their 70s and 80s.[57,58] More recently, there is emerging knowledge on components of innate immunity associated with AD pathology, as well as increasing discussion over the beneficial and detrimental effects of the immune response in AD. Another controversial topic is the start point of neuroinflammatory processes observed in AD brains, which raises the question as to whether inflammation is a cause or a consequence of AD pathology. Despite the initial assumption of their occurrence only in late stages of the disease, inflammatory changes in the CSF can be detected in MCI patients, revealing the possibility of involvement of the immune system at very early stages of AD.[59]

In a bid to answer the as yet unsolved questions regarding the *Janus face* of inflammation in AD, PET imaging is being used *in vivo* to trace markers of neuroinflammation with promising outcomes, as outlined below.[60]

Radiopharmaceuticals for imaging microglial activation. Neuroinflammatory changes are a part of AD pathology, and microglial activation in areas affected by neurodegeneration is a key brain tissue event.[61] Microglial activation occurring in early stages of AD dementia is associated with a significant elevation in the fractional area of reactive microglia following the formation of neuritic plaques comprising fibrillar Aβ.[62-65] Using a specific ligand for the 18-kDa translocator protein (TSPO), formerly called the peripheral benzodiazepine receptor (PBR), [11C]PK11195 quantification of microglial activation *in vivo* has been measured.[66] Probably due to its poor specific binding, initial [11C]PK11195 studies in AD showed negative results.[67] However, improvements in the [11C]PK11195 tracer, particularly with the utilization of its dextro-isomer, the radiotracer [11C]-(R)-PK11195, revealed increased sensitivity for detecting TSPO expression in parieto-temporal, entorhinal, and cingulate cortices of AD patients.[64] In [11C]PIB+ AD patients, high microglial activation has been observed, with an inverse correlation between cognition and microglial activity.[68] However, a recent study reported that the inclusion of a vascular component results in an amplified signal in AD patients,[69] suggesting that an increase in [11C]-(R)-PK11195 sensitivity signal modeling may be required.

Other TSPO radiopharmaceuticals have been designed aimed at improving pharmacokinetics and specificity. In this regard, preclinical studies involving radiotracers [18F]FEDAA1106 or [11C]AC5216 have shown promising results in AD-like transgenic models,[70,71] and [11C]DAA1106 PET imaging showed greater binding in AD patients compared to healthy control subjects.[72] Another recent clinical study using [18F]FEDAA1106 — a novel TSPO ligand with *in vitro* affinity superior to that of [11C]DAA1106[73] — showed widespread increases in MCI patients when compared to healthy controls, with these values predicting the conversion to AD dementia stage within a 5-year follow-up period.[74]

Although applications in studies of AD patients have not been performed, [11C]AC5216 has shown promising pharmacokinetic features and a higher affinity than [11C]PK11195 in healthy subjects.[75] However, the potential clinical applications of TSPO radiotracers are limited by the rs6971 polymorphism in the TSPO gene, which confers lower uptake in polymorphism carriers (~30%) in comparison to non-carriers.[76] Importantly, the cannabinoid receptor type 2 (CB2) was identified as a marker of microglial activation,[77] leading to greater attention to the endocannabinoid system. In this respect, preclinical studies of CB2 using [11C]A-836339 showed upregulation in a transgenic model displaying cerebral amyloidosis.[78]

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Radiopharmaceuticals for imaging reactive astrocytosis. In AD post-mortem tissue, the augmented expression of glial fibrillary acidic (GFAP) and astroglial S100B proteins is typically observed, indicating an increase in the number of reactive astrocytes.[79] Utilizing a ligand with a high affinity/specificity for monoamine oxidase B (MAO-B), an enzyme expressed primarily on the mitochondrial membrane of reactive astrocytes,[80,81] PET imaging using the carbon-11 labeled L-deprenyl ([^{11}C]DED) has revealed increased binding in patients with MCI, suggesting that astrocytosis is an early event in AD pathophysiology.[82]

PET BIOMARKERS OF NEURODEGENERATION

PET radiopharmaceuticals of tau pathology. Misfolding and aggregation of hyperphosphorylated tau deposition into NFTs has a key role in AD pathophysiology.[83,84] It is has been proposed that tau pathology propagates across brain circuits. NFTs have been associated with neuronal dysfunction, cell death, and cognitive impairment.[85] CSF levels of total tau (t-tau) and phosphorylated tau (p-tau) have been associated with disease severity,[86] with altered tau levels in the CSF being interpreted as surrogate markers of neurodegeneration in AD.[87] Despite this evidence, the clinical application of CSF tau as an AD biomarker has been further discussed elsewhere.[88]

CSF tau biomarkers are somewhat disadvantageous compared to imaging biomarkers given the need for a lumbar puncture. Moreover, CSF measurements provide global estimates of the disease process without any information regarding the topographic localization of NFT. Finally, the quantification of p-tau and t-tau protein varies significantly across centres.[89] Due to these features, imaging methods for NFT quantification are extremely important, particularly for assessing upcoming tau-based therapeutics. Given this scenario, several radiotracers characterized by high affinity for tau fibrils and with suitable kinetics have been developed, including the benzothiazole derivative [^{11}C]PBB3, the phenylquinoline derivatives—[^{18}F]THK-523, [^{18}F]THK-5105, and [^{18}F]THK-5117—and benzimidazole pyrimidine derivatives, such as [^{18}F]T807 and [^{18}F]T808.[90]

[^{11}C]PBB3 has shown to rapidly cross both the blood-brain barrier (BBB) and neuronal plasma membranes, binding to intraneuronal tau inclusions. *In vitro* and *ex vivo* autoradiographic studies have shown that [^{11}C]PBB3 produced specific, high-contrast labeling of neuronal tau inclusions in the brain stem of mice models expressing human tau pathological mutations. The same findings were reported with *in vitro* autoradiography and AD tissue, showing evident radiolabelling of fibrillar aggregates in specific regions of the hippocampus (including CA1), and the frontal cortex. Similarly, a clinical PET study using [^{11}C]PBB3 in probable AD patients revealed increased tracer binding in lateral temporal and frontal cortices, in line with the distribution of tau pathology at Braak stage V/VI, and with higher SUVRs correlating with lower memory scores. A slight increase in [^{11}C]PBB3 retention was also observed around the hippocampus of a control subject who showed decline on the Mini-Mental State Examination (MMSE), consistent with Braak stage III/IV or earlier.[91]

Aside from [^{11}C]PBB3, the fluorinated probes [^{18}F]T-807 and [^{18}F]T-808 are also potential tau ligands. An autoradiography study using AD brain tissue has shown that [^{18}F]T-807 exhibits strong binding to NFTs, with a selectivity estimate of 29-fold for tau relative to Aβ.[92] In addition, comparing double immunohistochemical staining of NFTs and Aβ on adjacent tissue sections, [^{18}F]T-807 autoradiography showed that tracer binding co-localized with immunoreactive NFTs, not with Aβ plaques.[93] In the case of [^{18}F]T-808, it also has a high affinity and good selectivity for NFTs over Aβ, which was indexed by autoradiographic studies.[94] In addition, [^{18}F]T-808 presents fast brain uptake followed by a rapid washout, suggesting low non-specific binding,[94] which is consistent with *in vivo* findings obtained using [^{18}F]T-807.[93] Moreover, the first human brain using [^{18}F]T-807 showed elevated SUVR in AD compared to MCI and healthy control subjects. Distinct patterns of tracer accumulation, in line with Braak staging, was observed across the frontal, temporal and parietal cortices, as well as in the hippocampus/entorhinal region.[92]

Another class of fluorinated probes includes the THKs, which have exhibited high affinity and selectivity for tau aggregates.[95] *In vitro* preliminary studies have reported [^{18}F]THK-523 binding affinity for tau fibrils, with subsequent autoradiographic analysis using AD medial temporal brain sections showing accumulation of [^{18}F]THK-523 in the pre- and pri-α layers of the entorhinal cortex and hippocampal CA1 region. Immunohistochemistry confirmed that these findings were consistent with the density of PHF-tau deposition.[96] Subsequently, histofluorescence and autoradiographic studies revealed that [^{18}F]THK-523 binding to NFTs co-localized with tau immunoreactivity, with no visible binding to Aβ plaques.[97] However, recent immunohistochemical and histofluorescence studies questioned [^{18}F]THK-523's future in both research and clinical settings due to very high non-specific white matter binding.[98] Moreover, preliminary clinical data have suggested that [^{18}F]THK-523 does not bind tau inclusions in non-AD tauopathies.[99]

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The second generation of THKs was developed, which includes [18F]THK-5105 and [18F]THK-5117. Binding studies conducted *in vitro* using [18F]THK-5105 and [18F]THK-5117 have found increased binding affinity to synthetic truncated tau (K18ΔK280) fibrils—comprising the four repeat regions (244-372) in the absence of lysine 280 (ΔK280)—with both tracers proving superior to [18F]THK-523. The selective binding capacity of these compounds was further examined using *in vitro* autoradiography and AD mesial temporal brain sections, showing increased accumulation of the radiotracer with particularly high binding in the Sommer's sector of the hippocampus, the parahippocampus, and the subiculum. These findings were confirmed through staining and immunohistochemistry, with reduced binding among healthy controls.[100] Additional assessment of [18F]THK-5105, conducted using [11C]PIB and AD hemibrain sections, observed dense accumulation of [18F]THK-5105 in tau-rich areas—including the hippocampus/parahippocampus, insula, cingulate gyrus and inferior and middle temporal gyri—with the pattern of tracer binding corresponding to the recognized distribution of tau pathology but not to that of Aβ or areas showing elevated retention of [11C]PIB. In addition, further studies of biodistribution conducted in normal mice showed profuse and rapid brain uptake and fast clearance, with the kinetics of both tracers superior to those reported for [18F]THK-523.[100-102]

[18F]FDG Radiotracer. The glucose analogue 2-deoxy-2-(18F)fluoro-D-glucose ([18F]FDG) PET has been utilized to assess cerebral glucose metabolism. [18F]FDG images have been interpreted as markers of neuronal activity[103] and synaptic density.[104] The typical AD metabolic signature consists of hypometabolism in the parietotemporal association, medial temporal, posterior cingulate and frontal cortices, with relative preservation of the visual cortex, primary sensory motor cortices, basal ganglia, thalamus, and cerebellum (Figure 2).[105-109] This hypometabolic signature is frequently observed in AD patients and in over 85% of pathologically-confirmed cases.[106] However, in atypical focal cortical syndromes of AD, topographic variants of hypometabolism have been identified. For example, compared to typical AD, patients with logopenic primary progressive aphasia presented with disproportionate left temporoparietal hypometabolism.[110] Patients with posterior cortical atrophy showed hypometabolism predominantly in occipito-parietal regions; some patients can present hypometabolism in the frontal eye fields.[111] Patients with early onset AD also show greater metabolic reductions, with hypometabolism from mild dementia comparable to that observed in late onset cases with severe dementia.[112] These findings are supported by studies showing more aggressive progression from patients with early onset AD[113] and, possibly, by the cognitive reserve theory.[114]

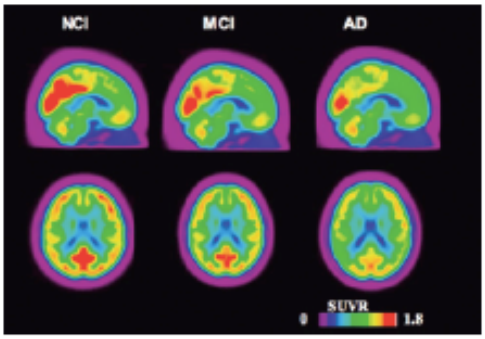

Figure 2. Representative PET [18F]FDG images acquired from a total of 103 individuals with normal cognition (NCI, N= 17), mild cognitive impairment (MCI, N=52) and dementia due to AD (AD, N= 27) obtained from a total of 103 structural MRI and [18F]FDG scans from the Alzheimer's Disease Neuroimage Initiative (ADNI) database. The hot color scale represents the magnitude of [18F]FDG standardized uptake value ratio (SUVR), proportional to glucose uptake. Note lower [18F]FDG SUVRs in MCI and AD as compared to controls. High SUVRs are particularly reduced in the posterior cingulate, precuneus and prefrontal (medial and dorsolateral) cortices. Imaging Methods: MRI images were corrected for non-linearity, classified (white, grey matter and CSF) and automatically segmented into cortical regions. Standard uptake value ratio (SUVR) images were calculated in PET native space using the cerebellum as a reference image. Images were subsequently resampled into the standard stereotaxic space and averaged using minctools.

In amnestic MCI (aMCI) patients, the pattern of hypometabolic changes usually occurs in brain regions classically affected in AD,[115] but to a lesser degree.[116-118] The patterns of brain metabolism in aMCI and non-amnestic MCI (naMCI) subjects are similar, however, aMCI has shown a decrease in medial temporal lobe metabolism and naMCI hypometabolism in the right prefrontal region.[119,120] Some authors report that the anterior hippocampal formation can contribute to differentiating MCI patients from healthy control subjects, although other data refutes this, with the partial volume effect in the metabolism of the hippocampal formation on [18F]FDG imaging constituting a complicating factor.[121-125] The posterior cingulate is the most relevant area for predicting conversion from MCI to AD, given that hippocampal hypometabolism is highly influenced by the atrophy observed on MRI and, after correcting

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for the partial volume effect, this finding is no longer supported.[117] [18F]FDG has limited clinical value in MCI patients due to the lack of specificity for AD pathophysiology. At a population level, however, subjects with MCI presenting a more marked or 'AD-like' pattern have been found to convert to dementia at higher rates,[126,127] with accuracies in the range of 75 to 100%.[128,129] The magnitude of hypometabolism in the parietal and posterior cingulate cortices in MCI is associated with memory decline.[130,131] As compared to decliners, stable MCI populations tend to exhibit hypometabolism restricted to the dorsolateral frontal cortex.[132,133]

In order to study the progression to MCI and AD among cognitively normal older individuals, [18F]FDG-PET has been used to predict cognitive decline with an accuracy approaching 80%.[121,122] Progressive reductions in PET glucose metabolism were observed years before the appearance of clinical symptoms, with reductions in the hippocampus preceding declines in cortical regions[134] in a clinicopathological study that evaluated cognitively normal individuals followed through MCI to pathologically-confirmed AD. The same metabolic changes have been noted in cognitively normal subjects homozygous for a susceptibility gene, the apolipoprotein E (APOE) ε4 allele,[120,135] asymptomatic carriers of genetic mutations associated with early onset familial AD,[128,129,136] and those with a maternal family history of AD, as compared to those with a paternal history or no family history of AD.[137] Ultimately, [18F]FDG will potentially prove of use in the characterization of a subgroup of patients exhibiting neurodegeneration in the absence of Aβ deposition.[138,139] These patients, classified in the category of "suspected non-amyloid pathophysiology (SNAP)", could suggest that the onset of neurodegeneration in AD may not depend on the accumulation of Aβ.[140]

IMAGING ALZHEIMER'S DISEASE PATHOPHYSIOLOGY IN EXPERIMENTAL MODELS

A miniaturized version of PET, termed microPET, has made non-invasive imaging of small animals possible, such as rats and mice. In addition, advances in genetic engineering have led to the development of diverse animal models harboring human pathological gene mutations, which are capable of mimicking amyloid and tau pathologies (for review see (141)). These models show progressive deposition of amyloid or tau, in parallel with significant cognitive decline, and are highly suited to longitudinal assessment with microPET.

To date, several studies have investigated AD pathophysiological events in rodent models with microPET (for review see (142)). However, few such studies have been conducted using a longitudinal design. Recently, the first prolonged longitudinal study with microPET evaluating amyloid was published and revealed non-linear patterns of amyloid deposition during full disease progression.[143] By contrast, to our knowledge, there are no longitudinal studies in the literature following tau pathology, and such studies are anxiously awaited by the AD community.[144] In this context, longitudinal studies associating these animal models and microPET imaging have high translational capability, which indicates that data collected can be rapidly translated to clinical studies. In addition, microPET longitudinal studies offer unprecedented opportunities for monitoring the effectiveness of innovative therapeutic strategies.

CONCLUSION

Predictions based on biomarkers indicate that AD pathophysiological abnormalities precede the onset of clinical symptoms by at least two decades. By obtaining earlier AD diagnosis, it will be possible to develop potential innovative therapies that may impact the natural progression of AD. In fact, several clinical studies testing potential new drugs are currently underway with amyloid and tau being the most promising pharmacological targets (for review see (145)). In this scenario, PET radiotracers and neuropathological features for these processes are crucial to determine amyloid and tau engagement and to assess treatment response in clinical trials. In keeping with this, radiopharmaceuticals for neuroinflammatory molecular imaging can equally contribute to the development of potentially effective anti-inflammatory interventions. Although the majority of PET studies in AD populations have focused on Aβ imaging, several 'non-amyloid' radiopharmaceuticals exist for evaluating neurodegeneration, neuroinflammation and perturbations in neurotransmission across the spectrum of AD, potentially contributing to improved therapeutic perspectives for AD (for review see (146)).

At present, neurology is experiencing a new era which encompasses imaging assessment for patients suspected of having AD. Thus, it is extremely important to accurately establish those patients who are candidates for performing a cerebral PET exam (e.g. amyloid imaging). In the case of amyloid positivity, this biomarker-based information reflects an elevated risk for AD, and health professionals should exercise caution when ordering this test in non-demented patients (e.g. healthy subjects and MCI patients). In the coming few years, when more effective therapies are likely to become available, amyloid imaging will be increasingly applied to identify

Table 2. PET imaging signatures in Alzheimer's disease.

Biological target	Radiotracers	Findings	Typical brain regions involved
Amyloid deposition	[^{11}C]PIB [^{18}F]Florbetapir [^{18}F]Florbetaben [^{18}F]Flutemetamol [^{18}F]NAV4694	Increased retention	Frontal cortex, medial and lateral posterior parietal cortices, precuneus Occipital cortex, lateral, temporal cortices and striatum
Tau pathology	[^{18}F]T807 [^{18}F]T808 [^{18}F]THK523 [^{18}F]THK5105 [^{18}F]THK5117 [^{11}C]PBB3	Increased retention	Frontal cortex, temporal cortex, parietal cortex and hippocampus/ entorhinal region
Glucose metabolism	[^{18}F]FDG	Low uptake	Parietotemporal association cortices, medial temporal cortex, posterior cingulate and frontal cortex (at later stages)
Neuroinflammation	[^{11}C]PK11195 [^{11}C]DAA1106 [^{18}F]FEDAA1106 [^{11}C]AC5216 [^{11}C]A836339 [^{11}C]L-deprenyl	Increased retention	Widespread retention within the whole brain

patients with early AD or at risk of developing AD; preferably in association with [^{18}F]FDG imaging in order to combine pathological (amyloid deposition) and metabolic (hypometabolism) information. Other radiotracers will also play a key role for research in AD (Table 2), especially in targeting novel therapies and for monitoring the response and efficacy of new drugs (e.g. tau and neuroinflammation).

Author contribution. Lucas Porcello Schilling collected and critically analysed literature, contributed with tables and wrote the paper; Eduardo R. Zimmer collected and critically analysed literature, contributed with figures and wrote the paper; Monica Shin collected and critically analysed literature and wrote the paper. Antoine Leuzy collected and critically analysed literature, contributed with figures and wrote the paper; Tharick A. Pascoal collected and critically analysed literature and wrote the paper; Andréa L. Benedet collected and critically analysed literature and wrote the paper. Wyllians V. Borelli collected and critically analysed literature and wrote the paper. André Palmini collected and critically analysed literature and wrote the paper; Serge Gauthier collected and critically analysed literature and wrote the paper; Pedro Rosa Neto collected and critically analysed literature and wrote and reviewed the paper.

REFERENCES

1. Cipriani G, Dolciotti C, Picchi L, Bonuccelli U. Alzheimer and his disease: a brief history. Neurol Sci. 2011;32(2):275-9.
2. Dahm R. Alzheimer's discovery. Curr Biol. 2006;16(21):R906-10.
3. Brookmeyer R, Johnson E, Ziegler-Graham K, Arrighi HM. Forecasting the global burden of Alzheimer's disease. Alzheimers Dement. 2007; 3(3):186-91.
4. Duyckaerts C, Delatour B, Potier MC. Classification and basic pathology of Alzheimer disease. Acta Neuropathol. 2009;118(1):5-36.
5. 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.
6. Jack CR, Jr., Albert MS, Knopman DS, et al. Introduction to the recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. Alzheimers Dement. 2011;7(3):257-62.
7. Dubois B, Feldman HH, Jacova C, et al. Research criteria for the diagnosis of Alzheimer's disease: revising the NINCDS-ADRDA criteria. Lancet Neurol. 2007;6(8):734-46.
8. Dubois B, Feldman HH, Jacova C, et al. Revising the definition of Alzheimer's disease: a new lexicon. Lancet Neurol. 2010;9(11):1118-27.
9. Sperling RA, Aisen PS, Beckett LA, 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.
10. Albert MS, DeKosky ST, Dickson D, 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.
11. McKhann GM, Knopman DS, Chertkow H, 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.
12. Jack CR, Jr., Knopman DS, Jagust WJ, et al. Hypothetical model of dynamic biomarkers of the Alzheimer's pathological cascade. Lancet Neurol. 2010;9(1):119-28.
13. Jack CR, Jr., Knopman DS, Jagust WJ, et al. Tracking pathophysiological processes in Alzheimer's disease: an updated hypothetical model of dynamic biomarkers. Lancet Neurol. 2013;12(2):207-16.
14. McGeer PL, McGeer EG. The amyloid cascade-inflammatory hypothesis of Alzheimer disease: implications for therapy. Acta Neuropathol. 2013;126(4):479-97.
15. Govoni S, Mura E, Preda S, et al. Dangerous Liaisons between Beta-Amyloid and Cholinergic Neurotransmission. Curr Pharm Des. 2014;20(15):2525-38.
16. Klunk WE, Engler H, Nordberg A, et al. Imaging brain amyloid in Alzheimer's disease with Pittsburgh Compound-B. Ann Neurol 2004;55(3):306-19.
17. Kemppainen NM, Aalto S, Wilson IA, et al. PET amyloid ligand [11C]PIB uptake is increased in mild cognitive impairment. Neurology. 2007; 68(19):1603-6.
18. Ikonomovic MD, Klunk WE, Abrahamson EE, et al. Post-mortem correlates of in vivo PiB-PET amyloid imaging in a typical case of Alzheimer's disease. Brain. 2008;131(Pt 6):1630-45.
19. Leuzy A, Zimmer ER, Hourling K, Rosa-Neto P, Gauthier S. Use of amyloid PET across the spectrum of Alzheimer's disease: clinical utility and associated ethical issues. Amyloid. 2014;21(3):143-8.
20. Leuzy A, Zimmer ER, Bhat V, Rosa-Neto P, Gauthier S. Imaging biomarkers for amyloid: a new generation of probes and what lies ahead. Int Psychogeriatr. 2014;26(5):703-7.
21. Okello A, Koivunen J, Edison P, et al. Conversion of amyloid positive and negative MCI to AD over 3 years: an 11C-PIB PET study. Neurology. 2009;73(10):754-60.
22. Pike KE, Savage G, Villemagne VL, et al. Beta-amyloid imaging and memory in non-demented individuals: evidence for preclinical Alzheimer's disease. Brain. 2007;130(Pt 11):2837-44.
23. Vandenberghe R, Van Laere K, Ivanoiu A, et al. 18F-flutemetamol amyloid imaging in Alzheimer disease and mild cognitive impairment: a phase 2 trial. Ann Neurol. 2010;68(3):319-29.
24. Wolk DA, Price JC, Saxton JA, et al. Amyloid imaging in mild cognitive impairment subtypes. Ann Neurol. 2009;65(5):557-68.
25. Jansen WJ, Ossenkoppele R, Knol DL, et al. Prevalence of cerebral amyloid pathology in persons without dementia: a meta-analysis. JAMA. 2015;313(19):1924-38.
26. Nordberg A, Carter SF, Rinne J, et al. A European multicentre PET study of fibrillar amyloid in Alzheimer's disease. Eur J Nucl Med Mol Imaging. 2013;40(1):104-14.
27. Forsberg A, Engler H, Almkvist O, et al. PET imaging of amyloid deposition in patients with mild cognitive impairment. Neurol Aging. 2008;29(10):1456-65.
28. Gomperts SN, Rentz DM, Moran E, et al. Imaging amyloid deposition in Lewy body diseases. Neurology. 2008;71(12):903-10.
29. Petrou M, Dwamena BA, Foerster BR, et al. Amyloid deposition in Parkinson's disease and cognitive impairment: a systematic review. Mov Disord. 2015;30(7):928-35.
30. Edison P, Rowe CC, Rinne JO, et al. Amyloid load in Parkinson's disease dementia and Lewy body dementia measured with [11C] PIB positron emission tomography. J Neurol Neurosurg Psychiatry. 2008;79(12):1331-8.
31. Claassen DO, Lowe VJ, Peller PJ, Petersen RC, Josephs KA. Amyloid and glucose imaging in dementia with Lewy bodies and multiple systems atrophy. Parkinsonism Relat Disord. 2011;17(3):160-5.
32. Johnson KA, Minoshima S, Bohnen NI, et al. Appropriate use criteria for amyloid PET: a report of the Amyloid Imaging Task Force, the Society of Nuclear Medicine and Molecular Imaging, and the Alzheimer's Association. J Nuclear Med. 2013;54(3):476-90.
33. Dubois B, Feldman HH, Jacova C, et al. Advancing research diagnostic criteria for Alzheimer's disease: the IWG-2 criteria. Lancet Neurol. 2014;13(6):614-29.
34. Soucy JP, Bartha R, Bocti C, et al. Clinical applications of neuroimaging in patients with Alzheimer's disease: a review from the Fourth Canadian Consensus Conference on the Diagnosis and Treatment of Dementia 2012. Alzheimers Res Ther. 2013;5(Suppl 1):S3.
35. Rowe CC, Villemagne VL. Amyloid imaging with PET in early Alzheimer disease diagnosis. Med Clin North Am. 2013;97(3):377-98.
36. Fodero-Tavoletti MT, Smith DP, McLean CA, et al. In vitro characterization of Pittsburgh compound-B binding to Lewy bodies. J Neurosci. 2007;27(39):10365-71.
37. Lockhart A, Lamb JR, Osredkar T, et al. PIB is a non-specific imaging marker of amyloid-beta (Abeta) peptide-related cerebral amyloidosis. Brain. 2007;130(Pt 10):2607-15.
38. Buckner RL, Snyder AZ, Shannon BJ, et al. Molecular, structural, and functional characterization of Alzheimer's disease: evidence for a relationship between default activity, amyloid, and memory. J Neurosci. 2005;25(34):7709-17.
39. Braak H, Braak E. Alzheimer's disease: striatal amyloid deposits and neurofibrillary changes. J Neuropathol Exp Neurol. 1990;49(3):215-24.
40. Brilliant MJ, Elble RJ, Ghobrial M, Struble RG. The distribution of amyloid beta protein deposition in the corpus striatum of patients with Alzheimer's disease. Neuropathol Appl Neurobiol. 1997;23(4):322-5.
41. Zhang S, Smailagic N, Hyde C, et al. (11)C-PIB-PET for the early diagnosis of Alzheimer's disease dementia and other dementias in people with mild cognitive impairment (MCI). Cochrane 2014;7:CD010386.
42. Sperling RA, Jack CR, Jr., Aisen PS. Testing the right target and right drug at the right stage. Sci Transl Med. 2011;3(111):111cm33.
43. Pascoal TA, Mathotaarachchi S, Mohades S, et al. Amyloid-beta and hyperphosphorylated tau synergy drives metabolic decline in preclinical Alzheimer's disease. Mol Psychiatry. 2016. doi: 10.1038/mp.2016.37.
44. Benedet AL, Labbe A, Lamay P, et al. Epistasis analysis links immune cascades and cerebral amyloidosis. J Neuroinflammation. 2015;12:227.
45. Mintun MA, Larossa GN, Sheline YI, et al. [11C]PIB in a nondemented population: potential antecedent marker of Alzheimer disease. Neurology. 2006;67(3):446-52.
46. Toledo JB, Bjerke M, Da X, et al. Nonlinear Association Between Cerebrospinal Fluid and Florbetapir F-18 beta-Amyloid Measures Across the Spectrum of Alzheimer Disease. JAMA Neurology. 2015;72(5):571-81.
47. Wolk DA, Grachev ID, Buckley C, et al. Association between in vivo fluorine 18-labeled flutemetamol amyloid positron emission tomography imaging and in vivo cerebral cortical histopathology. Arch Neurol. 2011;68(11):1398-403.
48. Clark CM, Schneider JA, Bedell BJ, et al. Use of florbetapir-PET for imaging beta-amyloid pathology. JAMA. 2011;305(3):275-83.
49. Syed YY, Deeks E. [(18)F]Florbetaben: A Review in beta-Amyloid PET Imaging in Cognitive Impairment. CNS Drugs. 2015;29(7):605-13.
50. Sabri O, Sabbagh MN, Seibyl J, et al. Florbetaben PET imaging to detect amyloid beta plaques in Alzheimer's disease: Phase 3 study. Alzheimers Dement. 2015;11(8):964-74.
51. Rowe CC, Pejoska S, Mulligan RS, et al. Head-to-head comparison of 11C-PiB and 18F-AZD4694 (NAV4694) for beta-amyloid imaging in aging and dementia. J Nuclear Med. 2013;54(8):880-6.
52. Fischer FU, Wolf D, Scheurich A, Fellgiebel A, Alzheimer's Disease Neuroimaging I. Altered whole-brain white matter networks in preclinical Alzheimer's disease. Neuroimage Clin. 2015;8:660-6.
53. Rosenberg PB, Wong DF, Edell SL, et al. Cognition and amyloid load in Alzheimer disease imaged with florbetapir F 18(AV-45) positron emission tomography. Am J Geriatr Psychiatry. 2013;21(3):272-8.
54. Thal DR, Beach TG, Zanette M, et al. [(18)F]flutemetamol amyloid positron emission tomography in preclinical and symptomatic Alzheimer's disease: Specific detection of advanced phases of amyloid-beta pathology. Alzheimers Dement. 2015;11(8):975-85.
55. Csakvari Z, Jonhagen ME, Forsberg A, et al. Clinical validation of 18F-AZD4694, an amyloid-beta-specific PET radioligand. J Nuclear Med. 2012;53(3):415-24.
56. Rowe CC, Villemagne VL. Brain amyloid imaging. J Nuclear Med Technol. 2013;41(1):11-8.
57. Ishii T, Haga S. Immuno-electron microscopic localization of immunoglobulins in amyloid fibrils of senile plaques. Acta Neuropathol. 1976;36(3):243-9.
58. Elikaimboom P, Stam FC. Immunoglobulins and Complement Factors in Senile Plaques - an Immunoperoxidase Study. Acta Neuropathol. 1982;57(2-3):239-42.
59. Brosseron F, Krauthausen M, Kummer M, Heneka MT. Body fluid cytokine levels in mild cognitive impairment and Alzheimer's disease: a comparative overview. Mol Neurobiol. 2014;50(2):534-44.
60. Zimmer ER, Leuzy A, Benedet AL, Breitner J, Gauthier S, Rosa-Neto P.

Tracking neuroinflammation in Alzheimer's disease: the role of positron emission tomography imaging. J Neuroinflammation. 2014;11:120.
61. McGeer EG, McGeer PL. Inflammatory processes in Alzheimer's disease. Prog Neuropsychopharmacol Biol Psychiatry. 2003;27(5):741-9.
62. Vehmas AK, Kawas CH, Stewart WF, Troncoso JC. Immune reactive cells in senile plaques and cognitive decline in Alzheimer's disease. Neurol Aging. 2003;24(2):321-31.
63. Akiyama H, Barger S, Barnum S, et al. Inflammation and Alzheimer's disease. Neurol Aging. 2000;21(3):383-421.
64. Cagnin A, Brooks DJ, Kennedy AM, et al. In-vivo measurement of activated microglia in dementia. Lancet. 2001;358(9280):461-7.
65. Versijpt JJ, Dumont F, Van Laere KJ, K, et al. Assessment of neuroinflammation and microglial activation in Alzheimer's disease with radiolabelled PK11195 and single photon emission computed tomography. A pilot study. Eur Neurol. 2003;50(1):39-47.
66. Papadopoulos V, Baraldi M, Guilarte TR, et al. Translocator protein (18kDa): new nomenclature for the peripheral-type benzodiazepine receptor based on its structure and molecular function. Trends Pharmacol Sci. 2006;27(8):402-9.
67. Groom GN, Junck L, Foster NL, Frey KA, Kuhl DE. PET of peripheral benzodiazepine binding sites in the microgliosis of Alzheimer's disease. J Nuclear Med. 1995;36(12):2207-10.
68. Edison P, Archer HA, Gerhard A, et al. Microglia, amyloid, and cognition in Alzheimer's disease: An [11C](R)PK11195-PET and [11C]PIB-PET study. Neurobiol Dis. 2008;32(3):412-9.
69. Tomasi G, Edison P, Bertoldo A, et al. Novel reference region model reveals increased microglial and reduced vascular binding of 11C-(R)-PK11195 in patients with Alzheimer's disease. J Nuclear Med. 2008;49(8):1249-56.
70. Higuchi M. Visualization of brain amyloid and microglial activation in mouse models of Alzheimer's disease. Curr Alzheimer Res. 2009; 6(2):137-43.
71. Maeda J, Zhang MR, Okauchi T, et al. In vivo positron emission tomographic imaging of glial responses to amyloid-beta and tau pathologies in mouse models of Alzheimer's disease and related disorders. J Neurosci. 2011;31(12):4720-30.
72. Yasuno F, Ota M, Kosaka J, et al. Increased binding of peripheral benzodiazepine receptor in Alzheimer's disease measured by positron emission tomography with [11C]DAA1106. Biol Psychiatry. 2008;64(10):835-41.
73. Wischik CM, Edwards PC, Lai RY, Roth M, Harrington CR. Selective inhibition of Alzheimer disease-like tau aggregation by phenothiazines. Proc Natl Acad Sci U S A. 1996;93(20):11213-8.
74. Yasuno F, Kosaka J, Ota M, et al. Increased binding of peripheral benzodiazepine receptor in mild cognitive impairment-dementia converters measured by positron emission tomography with [(1)(1)C]DAA1106. Psychiatry Res. 2012;203(1):67-74.
75. Miyoshi M, Ito H, Arakawa R, et al. Quantitative analysis of peripheral benzodiazepine receptor in the human brain using PET with (11)C-AC-5216. J Nuclear Med. 2009;50(7):1095-101.
76. Yoder KK, Nho K, Risacher SL, Kim S, Shen L, Saykin AJ. Influence of TSPO genotype on 11C-PBR28 standardized uptake values. J Nuclear Med. 2013;54(8):1320-2.
77. Horti AG, Gao Y, Ravert HT, et al. Synthesis and biodistribution of [11C] A-836339, a new potential radioligand for PET imaging of cannabinoid type 2 receptors (CB2). Bioorg Med Chem. 2010;18(14):5202-7.
78. Benito C, Tolon RM, Pazos MR, Nunez E, Castillo AI, Romero J. Cannabinoid CB2 receptors in human brain inflammation. Br J Pharmacol. 2008;153(2):277-85.
79. Varkhratsky A, Olabarria M, Noristani HN, Yeh CY, Rodriguez JJ. Astrocytes in Alzheimer's disease. Neurotherapeutics. 2010;7(4):399-412.
80. Fowler JS, MacGregor RR, Wolf AP, et al. Mapping human brain monoamine oxidase A and B with 11C-labeled suicide inactivators and PET. Science. 1987;235(4787):481-5.
81. Fowler JS, Volkow ND, Wang GJ, et al. Age-related increases in brain monoamine oxidase B in living healthy human subjects. Neurol Aging. 1997;18(4):431-5.
82. Carter SF, Scholl M, Almkvist O, et al. Evidence for astrocytosis in prodromal Alzheimer disease provided by 11C-deuterium-L-deprenyl: a multitracer PET paradigm combining 11C-Pittsburgh compound B and 18F-FDG. J Nuclear Med. 2012;53(1):37-46.
83. Jucker M, Walker LC. Pathogenic protein seeding in Alzheimer disease and other neurodegenerative disorders. Ann Neurol. 2011;70(4):532-40.
84. Spires-Jones TL, Stoothoff WH, de Calignon A, Jones PB, Hyman BT. Tau pathophysiology in neurodegeneration: a tangled issue. Trends Neurosci. 2009;32(3):150-9.
85. Iqbal K, Liu F, Gong CX, Alonso Adel C, Grundke-Iqbal I. Mechanisms of tau-induced neurodegeneration. Acta Neuropathol.2009;118(1):53-69.
86. de Calignon A, Polydoro M, Suarez-Calvet M, et al. Propagation of tau pathology in a model of early Alzheimer's disease. Neuron. 2012;73(4): 685-97.
87. Buerger K, Teipel SJ, Zinkowski R, et al. CSF tau protein phosphorylated at threonine 231 correlates with cognitive decline in MCI subjects. Neurology. 2002;59(4):627-9.
88. Augustinack JC, Schneider A, Mandelkow EM, Hyman BT. Specific tau phosphorylation sites correlate with severity of neuronal cytopathology in Alzheimer's disease. Acta Neuropathol. 2002;103(1):26-35.
89. Blennow K, Hampel H. CSF markers for incipient Alzheimer's disease. Lancet Neurol. 2003;2(10):605-13.
90. Zimmer ER, Leuzy A, Gauthier S, Rosa-Neto P. Developments in Tau PET Imaging. Can J Neurol Sci. 2014;41(5):547-53.
91. Maruyama M, Shimada H, Suhara T, et al. Imaging of tau pathology in a tauopathy mouse model and in Alzheimer patients compared to normal controls. Neuron. 2013;79(6):1094-108.
92. Chien DT, Bahri S, Szardenings AK, et al. Early clinical PET imaging results with the novel PHF-tau radioligand [F-18]-T807. J Alzheimers Dis. 2013;34(2):457-68.
93. Xia CF, Arteaga J, Chen G, et al. [(18)F]T807, a novel tau positron emission tomography imaging agent for Alzheimer's disease. Alzheimers Dement. 2013;9(6):666-76.
94. Zhang W, Arteaga J, Cashion DK, et al. A highly selective and specific PET tracer for imaging of tau pathologies. J Alzheimers Dis. 2012;31(3): 601-12.
95. Okamura N, Suemoto T, Furumoto S, et al. Quinoline and benzimidazole derivatives: candidate probes for in vivo imaging of tau pathology in Alzheimer's disease. J Neurosci. 2005;25(47):10857-62.
96. Harada R, Okamura N, Furumoto S, et al. Comparison of the binding characteristics of [18F]THK-523 and other amyloid imaging tracers to Alzheimer's disease pathology. Eur J Nucl Med Mol Imaging. 2013;40(1):125-32.
97. Fodero-Tavoletti MT, Okamura N, Furumoto S, et al. 18F-THK523: a novel in vivo tau imaging ligand for Alzheimer's disease. Brain. 2011; 134(Pt 4):1089-100.
98. Villemagne VL, Furumoto S, Fodero-Tavoletti MT, et al. In vivo evaluation of a novel tau imaging tracer for Alzheimer's disease. Eur J Nucl Med Mol Imaging. 2014;41(5):816-26.
99. Fodero-Tavoletti MT, Furumoto S, et al. Assessing THK523 selectivity for tau deposits in Alzheimer's disease and non Alzheimer's disease tauopathies. Alzheimers Res Ther. 2014;6(1):11.
100. Okamura N, Furumoto S, Harada R, et al. Novel 18F-Labeled Arylquinoline Derivatives for Noninvasive Imaging of Tau Pathology in Alzheimer Disease. J Nuclear Med. 2013;54(8):1420-7.
101. Johnson KA, Schultz A, Betensky RA, et al. Tau PET imaging in aging and early Alzheimer's disease. Ann Neurol. Ann Neurol. 2016;79(1): 110-9.
102. Marquie M, Normandin MD, Vanderburg CR, et al. Validating novel tau positron emission tomography tracer [F-18]-AV-1451 (T807) on postmortem brain tissue. Ann Neurol. 2015;78(5):787-800.
103. Sokoloff L, Reivich M, Kennedy C, et al. The [14C]deoxyglucose method for the measurement of local cerebral glucose utilization: theory, procedure, and normal values in the conscious and anesthetized albino rat. J Neurochem. 1977;28(5):897-916.
104. Rocher AB, Chapon F, Blaizot X, Baron JC, Chavoix C. Resting-state brain glucose utilization as measured by PET is directly related to regional synaptophysin levels: a study in baboons. Neuroimage. 2003; 20(3):1894-8.
105. Silverman DH, Small GW, Phelps ME. Clinical Value of Neuroimaging in the Diagnosis of Dementia. Sensitivity and Specificity of Regional Cerebral Metabolic and Other Parameters for Early Identification of Alzheimer's Disease. Clinical positron imaging : official journal of the Institute for Clinical PET. 1999;2(3):119-30.
106. Silverman DH, Small GW, Chang CY, et al. Positron emission tomography in evaluation of dementia: Regional brain metabolism and long-term outcome. JAMA2001;286(17):2120-7.
107. Mosconi L. Brain glucose metabolism in the early and specific diagnosis

of Alzheimer's disease. FDG-PET studies in MCI and AD. Eur J Nucl Med Mol Imaging. 2005;32(4):486-510.
108. Minoshima S, Giordani B, Berent S, Frey KA, Foster NL, Kuhl DE. Metabolic reduction in the posterior cingulate cortex in very early Alzheimer's disease. Ann Neurol. 1997;42(1):85-94.
109. Scholl M, Damian A, Engler H. Fluorodeoxyglucose PET in Neurology and Psychiatry. PET Clin. 2014;9(4):371-90.
110. Rabinovici GD, Jagust WJ, Furst AJ, et al. Abeta amyloid and glucose metabolism in three variants of primary progressive aphasia. Ann Neurol. 2008;64(4):388-401.
111. Nestor PJ, Caine D, Fryer TD, Clarke J, Hodges JR. The topography of metabolic deficits in posterior cortical atrophy (the visual variant of Alzheimer's disease) with FDG-PET. J Neurol Neurosurg Psychiatry. 2003;74(11):1521-9.
112. Kim EJ, Cho SS, Jeong Y, et al. Glucose metabolism in early onset versus late onset Alzheimer's disease: an SPM analysis of 120 patients. Brain 2005;128(Pt 8):1790-801.
113. Jacobs D, Sano M, Marder K, et al. Age at onset of Alzheimer's disease: relation to pattern of cognitive dysfunction and rate of decline. Neurology. 1994;44(7):1215-20.
114. Katzman R, Brown T, Thal LJ, et al. Comparison of rate of annual change of mental status score in four independent studies of patients with Alzheimer's disease. Ann Neurol. 1988;24(3):384-9.
115. Mosconi L, Brys M, Glodzik-Sobanska L, De Santi S, Rusinek H, de Leon MJ. Early detection of Alzheimer's disease using neuroimaging. Exp Gerontol. 2007;42(1-2):129-38.
116. Nestor PJ, Fryer TD, Smielewski P, Hodges JR. Limbic hypometabolism in Alzheimer's disease and mild cognitive impairment. Ann Neurol. 2003;54(3):343-51.
117. De Santi S, de Leon MJ, Rusinek H, et al. Hippocampal formation glucose metabolism and volume losses in MCI and AD. Neurobiol Aging. 2001;22(4):529-39.
118. Mosconi L, De Santi S, Li Y, et al. Visual rating of medial temporal lobe metabolism in mild cognitive impairment and Alzheimer's disease using FDG-PET. Eur J Nucl Med Mol Imaging. 2006;33(2):210-21.
119. Clerici F, Del Sole A, Chiti A, et al. Differences in hippocampal metabolism between amnestic and non-amnestic MCI subjects: automated FDG-PET image analysis. The quarterly journal of nuclear medicine and molecular imaging : official publication of the Italian Assoc Nucl Med. 2009;53(6):646-57.
120. Coutinho AM, Porto FH, Duran FL, et al. Brain metabolism and cerebrospinal fluid biomarkers profile of non-amnestic mild cognitive impairment in comparison to amnestic mild cognitive impairment and normal older subjects. Alzheimers Res Ther. 2015;7(1):58.
121. Mosconi L, De Santi S, Li J, et al. Hippocampal hypometabolism predicts cognitive decline from normal aging. Neurobiol Aging. 2008;29(5):676-92.
122. Mosconi L, Tsui WH, De Santi S, et al. Reduced hippocampal metabolism in MCI and AD: automated FDG-PET image analysis. Neurology. 2005;64(11):1860-7.
123. de Leon MJ, Convit A, Wolf OT, et al. Prediction of cognitive decline in normal elderly subjects with 2-[(18)F]fluoro-2-deoxy-D-glucose/poitron-emission tomography (FDG/PET). Proc Natl Acad Sci U S A. 2001;98(19):10966-71.
124. Samuraki M, Matsunari I, Chen WP, et al. Partial volume effect-corrected FDG PET and gray matter volume loss in patients with mild Alzheimer's disease. Eur J Nucl Med Mol Imag. 2007;34(10):1658-69.
125. Chételat G, Desgranges B, Landeau B, et al. Direct voxel-based comparison between gray matter hypometabolism and atrophy in Alzheimer's disease. Brain 2008;131(Pt 1):60-71.
126. Chételat G, Desgranges B, de la Sayette V, Viader F, Eustache F, Baron JC. Mild cognitive impairment: Can FDG-PET predict who is to rapidly convert to Alzheimer's disease? Neurology. 2003;60(8):1374-7.
127. Mosconi L, Perani D, Sorbi S, et al. MCI conversion to dementia and the APOE genotype: a prediction study with FDG-PET. Neurology. 2004;63(12):2332-40.
128. Drzezga A, Lautenschlager N, Siebner H, et al. Cerebral metabolic changes accompanying conversion of mild cognitive impairment into Alzheimer's disease: a PET follow-up study. Eur J Nucl Med Mol Imag. 2003;30(8):1104-13.
129. Herholz K, Nordberg A, Salmon E, et al. Impairment of neocortical metabolism predicts progression in Alzheimer's disease. Dement Geriatr Cogn Disord. 1999;10(6):494-504.
130. Anchisi D, Borroni B, Franceschi M, et al. Heterogeneity of brain glucose metabolism in mild cognitive impairment and clinical progression to Alzheimer disease. Arch Neurol. 2005;62(11):1728-33.
131. Herholz K, Salmon E, Perani D, et al. Discrimination between Alzheimer dementia and controls by automated analysis of multicenter FDG PET. Neuroimage. 2002;17(1):302-16.
132. Rossi S, Cappa SF, Babiloni C, et al. Prefrontal [correction of Prefontal] cortex in long-term memory: an "interference" approach using magnetic stimulation. Nat Neurosci. 2001;4(9):948-52.
133. Desgranges B, Baron JC, Eustache F. The functional neuroanatomy of episodic memory: the role of the frontal lobes, the hippocampal formation, and other areas. Neuroimage. 1998;8(2):198-213.
134. Mosconi L, Mistur R, Switalski R, et al. FDG-PET changes in brain glucose metabolism from normal cognition to pathologically verified Alzheimer's disease. Eur J Nucl Med Mol Imag. 2009;36(5):811-22.
135. Drzezga A, Grimmer T, Riemenschneider M, et al. Prediction of individual clinical outcome in MCI by means of genetic assessment and (18)F-FDG PET. J Nucl Med. 2005;46(10):1625-32.
136. Bateman RJ, Xiong C, Benzinger TL, et al. Clinical and biomarker changes in dominantly inherited Alzheimer's disease. New Engl J Med. 2012;367(9):795-804.
137. Mosconi L, Brys M, Switalski R, et al. Maternal family history of Alzheimer's disease predisposes to reduced brain glucose metabolism. Proc Natl Acad Sci U S A. 2007;104(48):19067-72.
138. Jack CR, Jr., Knopman DS, Weigand SD, et al. An operational approach to National Institute on Aging-Alzheimer's Association criteria for preclinical Alzheimer disease. Ann Neurol. 2012;71(6):765-75.
139. Knopman DS, Jack CR, Jr., Wiste HJ, et al. Short-term clinical outcomes for stages of NIA-AA preclinical Alzheimer disease. Neurology. 2012;78(20):1576-82.
140. Knopman DS, Jack CR, Jr., Wiste HJ, et al. Brain injury biomarkers are not dependent on beta-amyloid in normal elderly. Ann Neurol. 2013;73(4):472-80.
141. Götz J, Ittner LM. Animal models of Alzheimer's disease and frontotemporal dementia. Nat Rev Neurosci. 2008;9(7):532-44.
142. Zimmer ER, Parent MJ, Cuello AC, Gauthier S, Rosa-Neto P. MicroPET Imaging and transgenic models: a blueprint for Alzheimer's disease clinical research. Trends Neurosci. 2014;37(11):629-41.
143. Maier FC, Wahrl HF, Schmid AM, et al. Longitudinal PET-MRI reveals beta-amyloid deposition and rCBF dynamics and connects vascular amyloidosis to quantitative loss of perfusion. Nat Med. 2014;20(12):1485-92.
144. Zimmer ER, Leuzy A, Bhat V, Gauthier S, Rosa-Neto P. In vivo tracking of tau pathology using positron emission tomography (PET) molecular imaging in small animals. Transl Neurodegener. 2014;3(1):6.
145. Qian X, Hamad B, Dias-Lalcaca G. The Alzheimer disease market. Nat Rev Drug Discov. 2015;14(10):675-6.
146. Schilling LPL A, Zimmer ER, Gauthier S, Rosa-Neto P. Nonamyloid PET biomarkers and Alzheimer ' s disease : current and future perspectives. Future Neurol. 2014;9(6):597-613.

Discussão Artigo 3

O artigo intitulado “Imaging Alzheimer’s disease pathophysiology with PET” publicado no periódico Dementia & Neuropsychologia em 2016 é um artigo de revisão sobre a neuroimagem através do exame de PET na investigação da DA.

Neste artigo foram revisados os principais radiotraçadores utilizados na avaliação da DA, incluindo os relacionados ao acúmulo de amiloide. Neste artigo, apresentamos o PET como ferramenta na avaliação da DA como processo dinâmico, bem como sua aplicação na investigação das alterações patológicas, como acúmulo de amiloide, desde estágios pré-clínicos. Sob o ponto de vista clínico, revisamos especialmente o papel dos radiotraçadores de depósito de amiloide e de metabolismo de glicose como elementos importantes na abordagem diagnóstica da doença. Em casos específicos, como em pacientes com forma atípicas da DA, a avaliação da deposição de amiloide através do exame molecular fornece informações fundamentais para o diagnóstico preciso e que pode, por vezes, modificar de forma significativa o planejamento terapêutico para o paciente.

Através do desenvolvimento de biomarcadores de neuroimagem para DA, foram obtidos importantes avanços a respeito da progressão e dos processos relacionados com a doença. A incorporação progressiva desta ferramenta em grandes iniciativas como o ADNI tem permitido a quantificação e monitoramento de diversos aspectos patológicos da doença desde suas fases iniciais, trazendo a perspectiva do desenvolvimento de possíveis estratégias preventivas e terapêuticas especificas na DA.

ANEXO E – Documento de Submissão do Artigo Original

Manuscript #	SREP-16-46384
Current Revision #	0
Submission Date	11th November 16
Current Stage	Quality Check Started
Title	REGIONAL AMYLOID-β LOAD AND WHITE MATTER ABNORMALITIES CONTRIBUTE TO HYPOMETABOLISM IN ALZHEIMER'S DEMENTIA
Manuscript Type	Original Research
Corresponding Author	Dr. Pedro Rosa-Neto (pedro.rosa.neto@gmail.com) (McGill University)
Contributing Authors	Dr. Lucas Schilling , Dr. Tharick Pascoal , Dr. Eduardo Zimmer , Mr. Sulantha Mathotaarachchi , Miss Monica Shin , Dr. Carlos Rieder , Dr. Serge Gauthier , Dr. Andre Palmini
Authorship	Yes
Abstract	We investigated the association between amyloid-β deposition and WM integrity as a determinant of brain glucose hypometabolism across the AD spectrum.We assessed ninety-six subjects (27 cognitively normal (CN), 49 mild cognitive impairment (MCI); and 20 AD dementia) who underwent [18F]Fluorodeoxyglucose ([18F]FDG) and [18F]Florbetapir positron emission tomography (PET) as well as magnetic resonance imaging (MRI) with Diffusion Tensor Imaging (DTI).Among the regions with reduced fractional anisotropy (FA) in the AD group, we selected a voxel of interest (VOI) in the angular bundle bilaterally for subsequent analyses.Using voxel-based interaction models at voxel level, we tested whether the regional hypometabolism is associated with FA in the angular bundle and regional amyloid-β deposition.In the AD patients, [18F]FDG hypometabolism in the striatum, mesiobasal temporal, orbitofrontal, precuneus, anterior and posterior cingulate cortices were associated with the interaction between high levels of [18F]Florbetapir standard uptake value ratios (SUVR) in these regions and low FA in the angular bundle.We found that the interaction between, rather than the independent effects of, high levels of amyloid-β deposition and WM integrity disruption determined limbic hypometabolism in patients with AD.This finding highlights a more integrative model for AD, where the interaction between partially independent processes drives the disease progression.
Techniques	Physical sciences techniques [Imaging techniques];
Subject Terms	Health sciences/Neurology/Neurological disorders/Dementia/Alzheimer's disease Health sciences/Neurology/Neurological disorders/Neurodegenerative diseases/Alzheimer's disease
Competing Financial Interest	There is **NO** Competing Interest.
Applicable Funding Source	No Applicable Funding

ANEXO F - Carta de Aprovação da Comissão Científica

SIPESQ

Sistema de Pesquisas da PUCRS

PROPESQ

Código SIPESQ: 5962 Porto Alegre, 9 de dezembro de 2014.

Prezado(a) Pesquisador(a),

A Comissão Científica do INSTITUTO DO CÉREBRO DO RS da PUCRS apreciou e aprovou o Projeto de Pesquisa "Avaliação do impacto de alterações no PET na Conectividade Cerebral em Pacientes com Doença de Alzheimer" coordenado por ANDRE LUIS FERNANDES PALMINI. Caso este projeto necessite apreciação do Comitê de Ética em Pesquisa (CEP) e/ou da Comissão de Ética no Uso de Animais (CEUA), toda a documentação anexa deve ser idêntica à documentação enviada ao CEP/CEUA, juntamente com o Documento Unificado gerado pelo SIPESQ.

Atenciosamente,

Comissão Científica do INSTITUTO DO CÉREBRO DO RS

ANEXO G - Carta de Aprovação da Comissão de Ética

PONTIFÍCIA UNIVERSIDADE CATÓLICA DO RIO GRANDE DO SUL - PUC/RS

Plataforma Brasil

PARECER CONSUBSTANCIADO DO CEP

DADOS DO PROJETO DE PESQUISA

Título da Pesquisa: Avaliação do impacto de alterações no PET na Conectividade Cerebral em Pacientes com Doença de Alzheimer

Pesquisador: Andre Luis Fernandes Palmini

Área Temática:

Versão: 1

CAAE: 43241614.0.0000.5336

Instituição Proponente: UNIAO BRASILEIRA DE EDUCACAO E ASSISTENCIA

Patrocinador Principal: Financiamento Próprio

DADOS DO PARECER

Número do Parecer: 1.028.369

Data da Relatoria: 24/04/2015

Apresentação do Projeto:

O projeto visa comparar 50 imagens de pacientes dom Doenca de Alzheimer com 50 imagens de individuos sãos. As iamgens são provenientes do banco de dados da McGill com autorizacão do diretor do centro de Translational Neuroimaging Laboratory. Serão utilizados estudos de ressonancia magnetica e PET scan. O estudo visa avaliar as fibras da região parietal pela RM e a glicose e o amiloide por tecnicas modernas de PETcan. Anisotropia fracionada e difusibilidade media.

Objetivo da Pesquisa:

Verificar a aplicacão destas novas tecnicas no diagnostico precoce do Alzheimer.

Avaliação dos Riscos e Benefícios:

Sem riscos, face analise de banco de dados. Autorizacão e confidencialidade.

Comentários e Considerações sobre a Pesquisa:

Fundamental para os novos conhecimentos da doenca de Alzheimer.

Considerações sobre os Termos de apresentação obrigatória:

Adequados.

Recomendações:

PONTIFÍCIA UNIVERSIDADE CATÓLICA DO RIO GRANDE DO SUL - PUC/RS

Continuação do Parecer: 1.028.369

No titulo da trabalho não deve ser abreviatura como PET e sim o termo por extenso.

Conclusões ou Pendências e Lista de Inadequações:

Sem pendencias

Situação do Parecer:

Aprovado

Necessita Apreciação da CONEP:

Não

Considerações Finais a critério do CEP:

PORTO ALEGRE, 17 de Abril de 2015

Assinado por:
Rodolfo Herberto Schneider
(Coordenador)