UNIVERSIDADE D
COIMBRA

Rafael Soares Ferreira

Relatório de Estágio e Monografia intitulada "State-of-the-Art of Research and Development of Dendritic Cells Vaccines in the Treatment of Alzheimer's Disease" referentes à Unidade Curricular "Estágio", sob a orientação do Dr. Tiago Alexandre Gonçalves Parracho e da Professora Doutora Maria Teresa Cruz Rosete, apresentados à Faculdade de Farmácia da Universidade de Coimbra, para apreciação na prestação de provas públicas de Mestrado Integrado em Ciências Farmacêuticas.

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Julho 2021

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Coimbra, 16 de julho de 2021.

Rafael Soares Ferreira

(Rafael Soares Ferreira)

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Até Sempre!

"All we have to decide is what to do with the time that is given to us."

– Gandalf in The Lord of The Rings: The Fellowship of the Ring

Written by J. R. R. Tolkien

"– I´m getting older, my body is changing, and that's not something I'm gonna apologize for.

– I wish you would!"

– Trixie Mattel and Katya Zamolodchikova in UNHhhh Ep.24

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PARTE I

RELATÓRIO DE ESTÁGIO EM FARMÁCIA COMUNITÁRIA

ORIENTADO POR:

DR. TIAGO ALEXANDRE GONÇALVES PARRACHO

RESUMO

Após a formação adquirida durante o Mestrado Integrado em Ciências Farmacêuticas, o estudante deve realizar a Unidade Curricular de "Estágio Curricular". Sendo uma das áreas mais impactantes do mundo farmacêutico, a realização de um estágio em Farmácia de Oficina (ou Farmácia Comunitária), é obrigatória.

Desta forma, tive a possibilidade de estagiar na Farmácia Fonseca localizada na vila da Lousã, onde pude adquirir um vasto leque de conhecimentos sobre o funcionamento e gestão de uma farmácia e sobre o papel do farmacêutico de oficina como agente de saúde pública. Neste estágio é dada ao estudante uma oportunidade de perceber a sua futura intervenção como profissional de saúde multivalente e multicompetente.

Neste relatório é apresentada uma análise SWOT, de forma a evidenciar as forças, fraquezas, oportunidades e ameaças por mim notadas no decorrer deste estágio.

Palavras-Chave: Relatório de Estágio; Farmácia Comunitária; Farmácia Fonseca.

ABSTRACT

Following the training acquired during the Integrated Master's Degree in Pharmaceutical Sciences, students undertake the curricular unit "Curricular Internship". Being one of the most impactful areas of the pharmaceutical world, an internship in Community Pharmacy is mandatory.

Like so, I had the opportunity to be an intern in Farmácia Fonseca located in the village of Lousã, where I was able to obtain a vast range of knowledge about the functioning and management of a pharmacy and the role of a pharmacist as public health agent. In this internship, the student is given an opportunity to understand its future intervention as a multiskilled and multicompetent health care professional.

In the present report a SWOT analysis is presented, to demonstrate the strengths, weaknesses, opportunities, and threats that I noted in this internship.

Keywords: Internship Report; Community Pharmacy; Farmácia Fonseca.

ABREVIATURAS

ADIFA – Associação de Distribuidoras Farmacêuticas

ANF – Associação Nacional das Farmácias

APIFARMA – Associação Portuguesa da Indústria Farmacêutica

COVID-19 – *Coronavirus Disease – 2019*

DCI – Denominação Comum Internacional

GROQUIFAR – Associação de Grossistas de Produtos Químicos e Farmacêuticos

IMC – Índice de Massa Corporal

MICF – Mestrado Integrado em Ciências Farmacêuticas

MNSRM – Medicamento Não Sujeito a Receita Médica

MSRM – Medicamento Sujeito a Receita Médica

OF – Ordem dos Farmacêuticos

PDCA – Planear, Desenvolver, Confirmar, Atuar

SARS-CoV-2 – *Severe Acute Respiratory Syndrome – Coronavirus – 2*

SWOT – *Strengths, Weaknesses, Opportunities, Threats*

INTRODUÇÃO

No quinto ano do Mestrado Integrado Em Ciências Farmacêuticas (MICF), após 9 semestres de formação teórica, teórico-prática e prática, o plano de estudos contempla a realização de um estágio curricular em Farmácia Comunitária.

O estágio supracitado visa proporcionar ao aluno uma oportunidade de contacto com o mercado de trabalho da área farmacêutica, onde este pode aplicar os conhecimentos adquiridos nas diversas unidades curriculares frequentadas. É-nos, assim, possível compreender e fazer parte da missão do farmacêutico de oficina como agente de saúde pública, na resposta às mais diversas necessidades da população.

A oportunidade de desempenhar funções em Farmácia de Oficina é uma ligação importante e crucial entre o término da formação académica e o início do exercício da profissão farmacêutica, uma vez que permite aos estagiários uma melhor perceção do ato farmacêutico, das capacidades e vocações do próprio, e da carreira que pretende construir.

O meu estágio realizou-se na Farmácia Fonseca, Silva e Grade Lda., (Farmácia Fonseca) situada na interseção da Rua Conselheiro António Mesquita com a Avenida Coelho da Gama, no concelho da Lousã. A Farmácia Fonseca foi fundada em 1824, pelo farmacêutico António Correia da Costa, sendo, nos dias de hoje, a Dra. Maria Alice Neves Grade Poiares da Silva a proprietária e diretora técnica.

O estágio teve a duração de 810 horas, e realizou-se sob orientação do Dr. Tiago Alexandre Gonçalves Parracho, farmacêutico efetivo na Farmácia Fonseca há cerca de doze anos. Este período foi uma oportunidade extremamente valiosa para poder aprender e crescer com farmacêuticos mais experientes, onde tive a oportunidade de constatar o papel multivalente do farmacêutico como profissional e agente de saúde pública.

O presente relatório contempla uma análise SWOT (do inglês *Strengths*, *Weaknesses*, *Opportunities* e *Threats*) que visa relatar as atividades desenvolvidas neste estágio e dos conhecimentos adquiridos durante o mesmo.

ANÁLISE SWOT

1. PONTOS FORTES (*STRENGTHS*)

1.1. LOCALIZAÇÃO

A Farmácia Fonseca situa-se no centro da vila da Lousã, o que faz com que seja um ponto de fácil acessibilidade e que abrange uma quantidade considerável da população quer residente no concelho, quer de estadia temporária. A sua localização facilita a fidelização e confiança das pessoas na farmácia, o que encarga uma maior quantidade, mas também heterogeneidade de utentes, quer em termos de faixa etária, situação socioeconómica, estado de saúde, entre outros. A elevada afluência de diversos utentes na farmácia levou a uma necessidade de adaptação de postura, linguagem, e "plano de atendimento" consoante a pessoa que me encontrava a aconselhar.

1.2. PLANO DE ESTÁGIO

O estágio que realizei seguiu um plano de estágio estruturado e dinamizado para proporcionar um crescimento sólido e gradual do estudante como futuro farmacêutico (ver **Anexo I**). Desta forma, o meu plano de estágio contemplou três fases distintas: *back office*; transição *back* to *front*, e *front office*. Antes de iniciar o estágio propriamente dito, foi-me apresentada a equipa, as instalações, o modo e horário de funcionamento da farmácia, e as precauções relativas à atual pandemia.

Assim, iniciou-se a primeira fase, com duração de cerca de três semanas, que consistiu em: conhecer as cooperativas, armazenistas e fornecedores; receber e gerir encomendas e devoluções; armazenamento e arrumação de medicamentos, dispositivos médicos, e outros produtos; gestão de *stocks*, reservas, e prazos de validade; e ambientação com o programa SIFARMA2000® no contexto de *back office*. Esta fase é essencial para solidificar e agilizar o aconselhamento ao utente, uma vez que possibilita conhecer o local e modo de armazenamento dos diversos medicamentos e produtos, fazer a associação da Denominação Comum Internacional (DCI) dos princípios ativos ao medicamento de marca, e efetuar e gerir reservas de utentes.

Na fase de transição *back* to *front*, com duração de uma semana, realizei diversas tarefas que me prepariam para o atendimento ao utente. Algumas destas tarefas foram a realização do receituário e faturação, acompanhamento de atendimentos por parte de outros farmacêuticos e técnicos de farmácia, sensibilização e formação sobre os serviços prestados pela farmácia (como a medição de parâmetros bioquímicos e fisiológicos, o programa de troca de seringas, aconselhamento em dermofarmácia e

cosmética, veterinária, e maternidade, entre outros), e consolidação do uso do SIFARMA2000® no atendimento. Esta fase foi fundamental para conhecer melhor o tipo de receitas existentes e a maneira como cada uma deve ser analisada e dispensada, adquirir desenvoltura no desempenho dos diversos serviços prestados, e preparação para o atendimento ao utente.

Na última fase do estágio, foi-me dada autonomia no atendimento ao público onde passei a ter balcão próprio, junto ao balcão do Dr. Tiago Parracho. Aqui pude colocar em prática todos os conhecimentos e dicas que adquiri no MICF e nas semanas anteriores, juntamente com a formação contínua adquirida ao longo de todo o estágio.

1.3. EQUIPA

A equipa da Farmácia Fonseca é constituída por quatro farmacêuticos, três técnicas de farmácia e duas assistentes operacionais que trabalham em conjunto para que a farmácia se encontre sempre apta a satisfazer as necessidades dos seus utentes.

Destacam-se a grande amabilidade e empatia demonstrada por todos os elementos para com os estagiários e para com o público, sendo este, na minha opinião, um grande fator para a elevada confiança depositada pelos utentes nesta farmácia. A equipa trabalhou sempre com um espírito de entreajuda e profissionalismo, o que me permitiu esclarecer as minhas dúvidas e crescer durante o período de estágio.

1.4. *KAIZEN*™

Kaizen™ é um termo japonês que significa melhoria contínua e que quando aplicado ao local de trabalho consiste na evolução da administração e dos colaboradores de modo a obter, no caso de uma farmácia, uma melhor organização e facilidade no atendimento.[1] Na farmácia Fonseca a execução da metodologia *Kaizen*™ é realizada através da distribuição planeada de funções entre os colaboradores, a implementação de um plano PDCA (planear, desenvolver, confirmar e atuar), análise de indicadores de performance, entre outros.

Considero esta metodologia importante porque a farmácia fica com uma perspetiva abrangente do seu desempenho, podendo observar se os objetivos estabelecidos foram cumpridos, quer geral quer individualmente. Esta metodologia também permite à equipa analisar os aspetos que podem ser melhorados e o que pode ser feito de maneira diferente, de modo a otimizar o trabalho desenvolvido.

Esta metodologia impactou positivamente a minha experiência como estagiário, visto que me foram também dados objetivos e sugestões de melhoria. Desta forma, para além de ter a oportunidade de se integrado no trabalho da equipa, as metas estabelecidas

também estimularam a que houvesse da minha parte um maior esforço e dedicação diários. Assim, concluo que o *Kaizen*™ é uma metodologia eficaz, visto que é notória a melhoria continua na organização da farmácia e no atendimento ao utente.

1.5. SERVIÇOS FARMACÊUTICOS

A Portaria n.º 1429/2007 define os serviços farmacêuticos que uma farmácia pode prestar à população. Estes englobam o apoio domiciliário, administração de medicamentos e de vacinas não-incluídas nos plano nacional de vacinação, administração de primeiros socorros, utilização de meios auxiliares de diagnostico e terapêutica, e colaboração em programas de educação para a saúde.[2]

Durante o estágio tive a oportunidade de realizar a medição de parâmetros bioquímicos como a glicémia, colesterolémia e trigliceridémia, e de parâmetros fisiológicos como a pressão arterial, o peso e o índice de massa corporal (IMC). Estes serviços são realizados no Gabinete do Utente, visando permitir uma maior privacidade e um atendimento mais personalizado. A realização destes serviços é importante pois permite a sensibilização e acompanhamento do utente na prevenção e gestão de determinadas patologias e das suas consequências, como por exemplo, hipertensão arterial, aterosclerose, Diabetes *Mellitus*, obesidade entre outros.

Consequentemente, a integração e formação do estagiário na realização destes serviços não só amplia os seus conhecimentos, como permite a este assumir um papel ativo como agente de saúde pública.

1.6. VALORMED

A VALORMED é uma sociedade sem fins lucrativos e tem como principal foco de ação a gestão dos resíduos das embalagens vazias e medicamentos fora de uso. Foi criada em 1999 e é atualmente constituída pelos principais agentes da cadeia do medicamento como a Associação Portuguesa da Indústria Farmacêutica (APIFARMA), a Associação Nacional de Farmácias (ANF), a Associação de Grossistas de Produtos Químicos e Farmacêuticos (GROQUIFAR), e a Associação de Distribuidores Farmacêuticos (ADIFA).[3]

Esta sociedade visa implementar um sistema de gestão de resíduos medicamentosos de forma segura, o que traz benefícios, tais como a preservação do ambiente e a proteção da saúde pública.[3] Ao ser um profissional multivalente, o farmacêutico tem responsabilidade de promover o bem-estar do utente e da sociedade, a preservação do ambiente, e a educação e

sensibilização da população para o combate ao desperdício e poluição. Desta forma, a adesão da farmácia a esta iniciativa de recolha de medicamentos fora de uso é de extrema importância para consciencializar os utentes de que ao participarem neste processo estão a tomar um papel crucial num desenvolvimento sustentável. Na Farmácia Fonseca, os utentes têm uma elevada adesão devido ao trabalho de divulgação realizado por todos os colaboradores, incluindo estagiários.

1.7. RESPOSTA À PANDEMIA

Este estágio fica marcado por ter sido realizado durante a presente pandemia causada pelo *Severe Acute Respiratory Syndrome - Coronavirus - 2* (SARS-CoV-2). A farmácia respondeu de uma maneira extremamente segura e eficaz à presença deste vírus mediante a colocação de acrílicos em todos os balcões de atendimento, desinfeção constante de todos os espaços, produtos e equipamentos, uso obrigatório de máscara por parte de toda a equipa e utentes, marcação de espaços de espera dos utentes, e avaliação de sintomas por parte dos utentes e respetivo aconselhamento.

Aquando do início do meu estágio, a Farmácia Fonseca já se encontrava devidamente preparada para receber estagiários e utentes de uma forma organizada e segura. Assim, para além de me ter sentido seguro no estágio, não fui privado de realizar qualquer função inerente ao ato farmacêutico, como por exemplos a realização de testes bioquímicos.

2. PONTOS FRACOS (*WEAKNESSES*)

2.1. EQUIPAMENTO INFORMÁTICO

Na farmácia, o computador que utilizava para desempenhar as minhas funções apresentava falhas técnicas como, por exemplo, a demora na validação das receitas, a transição entre passos do atendimento e a emissão de recibos. Considero estas falhas um ponto fraco, pois o atendimento tornava-se moroso e com diversas quebras de fluidez, criando constrangimentos desnecessários.

Todas estas falhas obrigaram à necessidade de adotar ferramentas para minimizar o impacto destas no atendimento ao utente.

2.2. BALCÕES DUPLOS

Na farmácia, os postos de atendimento, apesar de distanciados, são duplos, onde em cada extremidade do posto se encontrava um membro da equipa. Na minha opinião, por

não existirem postos de atendimento individualizados ou distanciamento suficiente entre os farmacêuticos do mesmo balcão, a privacidade necessária durante o atendimento do utente encontra-se comprometida, prejudicando quer o meu desempenho neste, quer a experiência do utente.

2.3. NERVOSISMO E RECEIO

As elevadas responsabilidades subsequentes ao ato farmacêutico geraram algum nervosismo e inseguranças no desempenho das funções que me foram delegadas no atendimento ao utente. Estes sentimentos negativos tornaram os atendimentos demorados, com diversas interrupções e pouco satisfatórios para o utente. No entanto, foi-me sempre assegurado aconselhamento ou ajuda por parte de toda a equipa, em especial do meu orientador de estágio, que foram cruciais na superação destes obstáculos. Desta forma, esta situação teve um caráter temporário, na medida em que fui capaz de a superar e de apresentar uma postura, linguagem, confiança, e credibilidade corretas junto do utente.

2.4. FALTA DE CONFIANÇA DOS UTENTES

A inexperiência de um estagiário aliada à familiarização dos utentes para com a restante equipa fez com que, num período inicial da fase de atendimento, os utentes demonstrassem desconfiança ou desprezo nas minhas capacidades como profissional de saúde. Porém, com a habituação do público à minha presença na farmácia e por demonstrar um trabalho e aconselhamento de qualidade e confiante, permitiu que este problema fosse desparecendo, tornando-se raro com o decorrer do estágio.

3. OPORTUNIDADES (*OPPORTUNITIES*)

3.1. FORMAÇÃO CONTÍNUA

No decorrer de todo o estágio em farmácia comunitária, os diversos laboratórios, marcas, a ANF, a Ordem dos Farmacêuticos (OF), entre outras entidades disponibilizaram continuamente *webinars*, *workshops* e ações de formação a todos os profissionais de farmácia de oficina para que estes pudessem não só alargar o seu conhecimento, mas também manter-se atualizados sobre as diversas áreas de intervenção farmacêutica. Na minha opinião, esta é uma das melhores ferramentas que os farmacêuticos têm ao seu dispor, uma vez que lhes permite estarem atualizados

sobre os mais diversos temas e melhorarem o aconselhamento farmacológico e não-farmacológico aos utentes.

Considerei que a minha formação académica foi extremamente importante, mas que a constante procura por parte do farmacêutico para atualizar ou adquirir conhecimentos na área da saúde é uma ferramenta crucial e inevitável para que o seu papel de excelência como agente multivalente de saúde pública não só se mantenha, mas também que evolua continua e progressivamente.

3.2. AUTONOMIA E RESPONSABILIDADE

Desde o início do estágio na Farmácia Fonseca que me foram delegadas diversas tarefas nas diferentes fases do mesmo, como por exemplo, a verificação do receituário, o atendimento autónomo, o fecho de caixa, a receção e gestão de encomendas e devoluções, a verificação e notificação de prazos de validade, entre outras. Todas as tarefas foram guiadas e coordenadas pelo orientador de estágio. No entanto, foi-me sempre dada bastante autonomia no desempenho de funções, o que me providenciou espaço para estar em contacto direto com as mais variadas funções do farmacêutico de oficina.

No meu ponto de vista, toda a autonomia e versatilidade que tive de desenvolver e as responsabilidades que me foram incutidas possibilitaram o meu crescimento como profissional de saúde. Este crescimento e evolução deram-se na medida em que pude executar, errar, e aprender com obstáculos e dificuldades encontradas.

3.3. O FARMACÊUTICO E OS PÓS-ATENDIMENTO

Na Farmácia Fonseca a quantidade de utentes fidelizados é bastante elevada, o que levava a que já houvesse um certo conhecimento de causa por parte do farmacêutico em relação a cada utente. Assim, era de extrema importância acompanhar o utente após o atendimento estar concluído.

No decorrer de todo o estágio, especialmente na fase de *front office*, foi frequente o contacto posterior ao atendimento com um utente para fazer acompanhamento de uma situação passada. Este acompanhamento era feito através de contacto telefónico ou quando o utente se dirigia novamente à farmácia, onde era questionado sobre a eficácia das medidas aconselhadas no atendimento anterior. O desfecho deste acompanhamento pós-aconselhamento poderia ser: referenciação ao médico de família ou de especialidade; a continuação, interrupção, ou mudança do tratamento; o reforço das

medidas não farmacológicas; e, no caso de resolução do problema, a tomada de medidas para prevenção do reaparecimento do mesmo ou outros problemas associados.

Com a experiência adquirida durante o estágio, foi notório o impacto do acompanhamento pós-atendimento na fidelização dos utentes à farmácia, no aconselhamento informado ao utente, e na manutenção da saúde do utente e da população.

4. AMEAÇAS (*THREATS*)

4.1. OUTRAS FARMÁCIAS

Na vila da Lousã existem três farmácias com uma relativa proximidade entre si. É, assim, notória não só a forte concorrência entre as três farmácias, mas também a necessidade de um esforço acrescido por parte da equipa para a fidelização de utentes. Algumas das estratégias adotadas para combater esta ameaça são: a entrega gratuita de medicamentos ao domicílio; a cooperação entre as três farmácias no caso de rutura de *stocks* de medicamentos; aconselhamentos adequados e adaptados a cada utente; e a relação interpessoal entre os utentes e os farmacêuticos e técnicos de farmácia.

Por esta razão, e por estar incluído nos objetivos da equipa, tive de ter um papel ativo na competitividade da farmácia. Assim, tive de estabelecer padrões elevados na qualidade do atendimento e procurar suprir eficaz e rapidamente às necessidades dos utentes, de forma a preservar a excelência do atendimento, a que os utentes já se encontram habituados.

4.2. ESTABELECIMENTO DE VENDA DE MNSRM

O Decreto-Lei n.º 124/2005 de 16 de agosto veio possibilitar a venda de medicamentos e outros produtos de saúde em estabelecimentos específicos, denominados comummente por parafarmácias.[4] A existência de um estabelecimento de venda de medicamentos não sujeitos a receita médica (MNSRM) pertencente ao grupo Sonae no concelho da Lousã apresenta diversos desafios às restantes farmácias, principalmente à Farmácia Fonseca por ser a que se encontra mais perto deste. Além da competitividade na venda de medicamentos não sujeitos a receita médica, produtos de dermofarmácia e cosmética, entre outros, este estabelecimento possui técnicas de fidelização associadas a estabelecimentos comerciais do mesmo grupo, também presentes na vila da Lousã.

Além da ameaça de caráter financeiro, está também presente a frequente inexistência de farmacêuticos nestes estabelecimentos, o que compromete um aconselhamento correto e adequado aos utentes, pondo em causa a saúde pública.

Considero que a existência deste estabelecimento impactou negativamente o meu estágio, uma vez que os utentes descartavam o aconselhamento informado para se dirigirem ao referido estabelecimento e/ou possuíam conhecimentos errados sobre determinados produtos em medicamentos.

4.3. DESINFORMAÇÃO

Nos dias de hoje, o acesso à informação sobre saúde está mais facilitado devido às novas tecnologias e ao acesso à *internet*. No entanto, a informação nem sempre provém de fontes fidedignas, como é exemplo a informação presente nas redes sociais, nos meios de comunicação social e a proveniente de experiências pessoais. É usual que os utentes, após ouvir o aconselhamento farmacêutico, o questionem.

Os utentes dirigiam-se à farmácia com um "autodiagnóstico" formado, baseando-se em experiências próprias e/ou de conhecidos seus. Frequentemente, o requisito de medicamentos (maioritariamente de medicamentos sujeitos a receita médica (MSRM)) acontecia em situações de dores agudas onde requisitavam paracetamol em comprimidos de 1000mg; furosemida e/ou alopurinol para edemas dos membros inferiores e superiores; e ácido acetilsalicílico em comprimidos de 100mg porque, passo a citar, "já os antigos diziam"; entre outros.

A pandemia da *Coronavirus Disease 2019* (COVID-19) veio agravar o problema da desinformação dos utentes, como foi caso a ida à farmácia a solicitar Hidroxicloroquina para profilaxia desta doença. Estas situações recorrentes durante o estágio levaram-me a sentir uma descredibilização do papel do farmacêutico como profissional de saúde por parte dos utentes.

4.4. IMPACTOS DA PANDEMIA

A pandemia e o dever de confinamento imposto pelo governo no Decreto do Presidente da República n.º 6-B/2021 resultaram numa alteração do quotidiano da sociedade e esse impacto foi sentido na farmácia.[5]

Esta situação resultou: numa diminuição da afluência da população à farmácia, só se dirigindo a esta em casos de extrema necessidade; num decréscimo do poder de compra dos utentes, resultando numa grande dificuldade de realizar *cross-selling* ou de os utentes aderirem ao que lhes era aconselhado; e no uso obrigatório de máscara e da

presença de acrílicos nos balcões, visto que formaram uma barreira de comunicação entre o utente e o farmacêutico.[6]

Assim, considero que estagiei numa situação social e económica anómala, o que levou a uma diminuição na proximidade da relação interpessoal com o utente. Notoriamente, este é um aspeto do atendimento farmacêutico que eu não tive oportunidade de experienciar por completo.

CASOS PRÁTICOS

CASO 1 – CANDIDÍASE VAGINAL

Utente S.D. do sexo feminino, de aproximadamente 45 anos, dirige-se à farmácia com queixas de desconforto pélvico, vermelhidão e prurido. Refere que não é uma situação comum e que não sabe qual a etiologia do problema.

Após lhe ter colocado algumas questões percebi que a senhora pratica relações sexuais desprotegidas com o marido, não ingere quantidades suficientes ou recomendáveis de água, e tinha finalizado há alguns dias a toma de amoxicilina 875mg + ácido clavulânico 125mg em regime posológico de 1-0-1 durante 8 dias, depois de ter sido submetida a uma cirurgia dentária. Desta forma, orientei o atendimento de forma a levar a utente a perceber que estava perante uma situação de desregulação da flora vulvovaginal.

Assim, para uma resolução mais rápida do problema e para prevenção de uma recidiva, comecei por recomendar algumas medidas não farmacológicas: consumo diário de 1,5L a 2,5L de água; evitar usar roupa apertada ou de material sintético, devendo optar por roupa interior de algodão; fazer uma correta higienização de toda a zona pélvica, com especial cuidado de secar bem toda essa zona; evitar o uso de compostos com elevado teor de detergentes; e usar preservativo e lubrificante no caso de ter relações sexuais com o marido, e tentar urinar após as mesmas. Desta forma cedi à utente Gyno-Canestest®, uma vez que os sintomas relatados não são específicos para despiste entre vaginose bacteriana e candidíase[7]. Foi pedido à utente que voltasse à farmácia assim que executasse o teste e soubesse o resultado.

A utente S.D. voltou no dia seguinte à farmácia queixando-se que o problema persistia e que sentia prurido e desconforto mais intenso e profundo e apresentava agora corrimento vaginal, sobre o qual afirmou, após lhe ter sido perguntado, ter coloração esbranquiçada e não possuir odor fétido. Trazia também consigo o resultado do teste que se encontrava negativo para vaginose bacteriana.

Desta forma, caracterizei a situação como uma candidíase vaginal, tendo sido aconselhada a aplicação interna e externa de Gino-Canesten® creme de clotrimazol a 1% uma vez por dia, durante 6 dias.[8] Juntamente, reforcei as medidas não farmacológicas e recomendei o gel Gyn-8 Uriage® indicado para a limpeza da zona íntima feminina em situações agudas de desconforto e/ou em concomitância com tratamentos anti-fúngicos.[9]

CASO 2 – CRISE HEMORROIDÁRIA

Utente A.M. do sexo masculino, 45 anos de idade dirige-se à farmácia com queixas de dor a defecar e desconforto e prurido ao manter-se sentado. O utente revela ser a segunda vez que esta situação lhe acontece. No entanto, desta vez apresenta pequenos vestígios de sangue vermelho escarlate nas fezes, marcadamente aquando da higienização da zona anal, que relata ser, agora, um processo bastante doloroso.

Após algumas questões sobre o seu estilo de vida, o utente relata que passa muito tempo sentado (devido à sua profissão) e apresenta uma alimentação desequilibrada (ainda que ingira bastante água) e uma rotina sedentária. Pelos factos recolhidos, dirigi o meu aconselhamento para um problema de hemorroidas não-complicadas.

Assim, comecei o aconselhamento por explicar ao utente em que consistia o problema que ele apresentou, referindo as suas possíveis origens, podendo ser a alimentação, a falta de exercício físico, entre outras.

O senhor A.M. aparentou ter entendido o problema que tinha, pelo que procedi à explicação das medidas não-farmacológicas cruciais para a superação da crise hemorroidária, nomeadamente: ingestão de bastante água; adoção de uma alimentação rica em fibras, vegetais e frutas em detrimento do consumo de gorduras, comidas picantes ou fortemente temperadas, e alimentos ricos em açúcar; evitar o consumo de bebidas alcoólicas e café; praticar atividade física, tanto quanto possível; e fazer a limpeza da zona anal com um agente de lavagem próprio e água fria.

No aconselhamento foi dispensado NeoFitoroid® creme lavante para higienização da zona afetada, uma vez que tem complexos vegetais com ação detergente e lenitiva indicados para problemas de hemorroidas.[10] Para alívio do problema e dos respetivos sintomas foi-lhe cedida a pomada Procto-Glyvenol® em regime posológico 1-0-1 durante uma semana e reduzir, depois, a posologia para uma vez ao dia. Esta pomada contém tribenosido, que reduz a permeabilidade capilar, melhora o tónus vascular e tem ação anti-inflamatória (aliviando a dor, o inchaço e a vermelhidão); e lidocaína que é um anestésico local (aliviando a comichão, a dor e o ardor).[11] Acrescentando ao facto de não ser a primeira vez que apresenta sintomas característicos de crise hemorroidária, foi-lhe também cedido Daflon® em comprimidos de 1000mg para tomar 3 vezes ao dia durante a primeira semana, seguindo-se de 2 vezes ao dia durante a segunda semana, e reduzir para 1 vez por dia nas semanas subsequentes. Daflon® consiste na fração micronizada de bioflavonoides que aumentam o tónus vascular e a resistência capilar (diminuindo o sangramento, a dor e o desconforto).[12,13]

CASO 3 – DISTÚRBIOS DO SONO

Utente G.L. do sexo masculino de 22 anos dirige-se à farmácia com queixas quanto à qualidade do seu sono, relatando dificuldade e demora em adormecer. Afirma também não sentir que tem um sono descansado e que durante o dia apresenta sinais de cansaço e fadiga física e mental. O utente relata também estar em época de avaliações, o que lhe causa alguma ansiedade e agitação diárias.

Sendo assim, comecei por questionar o utente quanto aos seus hábitos diários e a sua rotina de noite. O utente relatou que está bastante tempo em contacto com ecrãs durante o dia, bebe 3 cafés por dia, não consome bebidas alcoólicas regularmente, e é fumador. Além disso, G.L. pratica exercício físico regularmente e diz ter uma alimentação minimamente equilibrada.

Posto isto, foram dadas ao utente diversas dicas para melhorar a sua higiene do sono, sendo elas: estabelecer um horário para adormecer e acordar todos os dias; evitar estar à frente de ecrãs 30 minutos antes e na hora de ir dormir; separar a zona de estudo e trabalho do quarto; evitar o consumo de tabaco, cafeína, bebidas alcoólicas e alimentos açucarados a partir da tarde; não comer nem beber em grande quantidade à noite; e dormir com luzes apagadas e temperatura adequada.[14]

Para corrigir a situação, foi cedido ao utente Dormidina® (doxilamina) comprimidos de 25mg para tomar 30 minutos antes da hora definida para se deitar. A doxilamina é um anti-histamínico com efeitos anticolinérgicos moderados e está indicada para reduzir o tempo necessário para adormecer e aumentar a profundidade e duração do mesmo.[15] Para auxiliar com o *stress* diário foi também aconselhado Valdispert® comprimidos de 125mg na posologia de 3-3-3, uma vez que a *Valeriana officinalis* nesta posologia está indicada na redução dos sintomas de *stress* e ansiedade.[16]

CONCLUSÃO

O estágio curricular em Farmácia Comunitária é uma excelente oportunidade dada ao futuro farmacêutico para, sob acompanhamento e orientação de profissionais mais experientes, entrar em contacto com a área de atendimento às necessidades de saúde da população e exercer funções de agente de saúde pública. Este estágio permite ao estudante desenvolver as suas capacidades e conhecimentos técnicos, científicos, socias e interpessoais inerentes ao ato farmacêutico.

O estágio de cerca de 6 meses na Farmácia Fonseca permitiu-me desenvolver e pôr em prática todos os conhecimentos adquiridos nos 5 anos do MICF, tendo este sido uma ferramenta crucial no meu crescimento como profissional de saúde e agente da ciência. Ainda que apenas futuro farmacêutico, foi notório que o trabalho feito por mim no decorrer deste estágio asseverou o papel do farmacêutico na saúde física, mental e social da população. Foi também de extrema gratificação ter ficado a conhecer os processos e mecanísticas envolvidos por trás do atendimento, quer na gestão da farmácia quer na gestão de toda a equipa.

Dado o término deste estágio, assumo uma posição confiante em relação ao que aprendi e ao que ainda vou ter oportunidade de aprender como profissional de saúde e como forte interveniente na melhoria da qualidade de vida da população.

Finalizo o presente relatório com um enorme agradecimento a toda a equipa da Farmácia Fonseca por me ter recebido e ensinado de uma forma tão profissional e humana. Agradeço especialmente ao Dr. Tiago Parracho e à Dra. Joana Silva por todos os conhecimentos, ferramentas e dicas que me transmitiram.

ANEXO

ANEXO 1 – TEMPLATE DE PLANO DE ESTÁGIO

PLANO ESTÁGIO Orientador - Tiago Parracho Estagiário - Rafael Soares Ferreira Data - 18/1/2021 a 18/6/2021	VERIFICAÇÃO	RUBRICA ESTAGIÁRIO	RUBRICA COORDENADOR
RECEPÇÃO-INTEGRAÇÃO			
Conhecer o espaço físico			
Cacifos			
Copa			
Organograma da equipa			
Plano Contingência Covid			
Horários			
Kaizen			
APROVISIONAMENTO			
Contactos			
Receção encomendas			
Preços/margens			
Stocks			
Validades			
Arrumação			
Reservas/Pedidos			
Devoluções			
Listagens de irregularidades			
Notas de devolução			
Realização de encomendas			
Produtos sem consumo			
Exceções de arrumação			
CARTÃO SAUDA			
Rebate pontos			
FATURAÇÃO			
Diplomas e planos de comparticipação			
Organização receituário			
Fecho faturação			
Contabilidade documentos mensais			
VALIDADES			
PSICOTRÓPICOS			
MANIPULADOS			
Preparação/preço/rótulo			
Topitek			
RECICLAGEM-VALORMED			
TROCA SERINGAS			
LINEARES			
Dermofarmácia			
Puericultura			
Higiene			
Saúde oral			
Dietética			
Suplementos Alimentares			

Nutrição			
Capilares			
Material penso			
Incontinência			
Veterinária			
Insuficiência Venosa – meias, medidas			
Homem			
Ortopedia			
SERVIÇOS FARMACÊUTICOS			
Glicémia/ Aparelhos/canetas insulina			
libre			
Colesterol			
TA			
Peso/Altura/IMC			
Administração vacinas/ injetáveis			
CONSULTAS			
FORMAÇÃO			
Plano formação			
Fluxogramas			
CDs			
Revistas/ Folhetos			
Webinars			
CARTÕES FIDELIDADE			
MONTRAS			
ATENDIMENTO			
Instrução trabalho/ script atendimento			
Sigilo profissional			
SIFARMA2000®			
Código colaborador			
Material escritório/Consumíveis			
Caixa			
Receção do utente e despedida			
Postura/SORRISO/PACIÊNCIA			
Linguagem			
Receitas eletrónicas/Manuais			
Venda MSRM e MNSRM			
Vendas suspensas			
Créditos			
Medidas não farmacológicas			
Posologia – oral, escrita			
Cross selling			
Fim dia – material, balcão, caixa			
Rentabilidade/Laboratórios			
Reação adversa aos medicamentos			
Faltas medicação – armazém – outras farmácias – domicílio – resolver situação			
PROATIVIDADE			
PROJETO DE MELHORIA			
Balcão, arrumação, atendimento, …			

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PARTE II

STATE-OF-THE-ART OF RESEARCH AND DEVELOPMENT OF DENDRITIC CELLS VACCINES IN THE TREATMENT OF ALZHEIMER'S DISEASE

ADVISED BY:

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RESUMO

A Doença de Alzheimer é uma doença neurodegenerativa que contempla diversos mecanismos e fases que levam a morte neuronal, disfunção cognitiva, e demência. Esta doença é principalmente caracterizada pela presença de: placas contendo oligómeros e fibrilhas de Aβ; emaranhados neurofibrilares de proteína tau hiperfosforilada; morte neuronal; e inflamação. Devido aos resultados sub-ótimos das terapêuticas atuais, os investigadores têm procurado respostas noutras estratégias, como a imunoterapia. A crescente evidência relativa ao papel das células dendríticas (DCs) em doenças neurodegenerativas e os resultados positivos alcançados com vacinas à base de DCs noutras patologias levou os investigadores a focarem-se no uso de DCs para desencadear respostas imunes e gerar anticorpos específicos para combater oligómeros de Aβ, um dos maiores *hallmarks* da Doença de Alzheimer. Os estudos a focarem-se nesta abordagem terapêutica começaram em 2008 e mostraram resultados promissores na função cognitiva. Isto pode querer dizer que se encontrou um novo caminho para alcançar uma terapêutica eficaz para a Doença de Alzheimer.

Palavras-Chave: Doença de Alzheimer; Demência; Células Dendríticas; Vacina; Imunoterapia.

ABSTRACT

Alzheimer's Disease is a neurodegenerative disease that contemplates several mechanisms and phases that lead to neuronal death, cognitive impairment, and dementia. This disease is mainly characterized by the presence of: plaques containing Aβ oligomers and fibrils; neurofibrillary tangles of hyperphosphorylated tau protein; neuronal death, and inflammation. Due to the underwhelming outcomes of current therapeutic approaches, researchers have been searching for other strategies, such as immunotherapy. The growing evidence concerning the role of dendritic cells (DCs) in neurodegenerative diseases and the positive results achieved with DCs-based vaccines in other pathologies led researchers to investigate the use of DCs to elicit immune responses and generate specific antibodies to fight Aβ oligomers, one of the most relevant hallmarks of Alzheimer's Disease. Studies focusing on this therapeutic approach began in 2008 and have shown extremely promising results in cognitive function. This could mean that we found a new path to achieve an effective therapy for AD.

Keywords: Alzheimer's Disease; Dementia; Dendritic Cells; Vaccine; Immunotherapy.

ABBREVIATIONS

ABCA1 – ATP-Binding Cassette Transporter A1

AD – Alzheimer's Disease

ADAD – Autosomal Dominant Alzheimer's Disease

ADD – Alzheimer's Disease and Dementia

ADI – Alzheimer's Disease International

AEP – Asparaginyl Endopeptidase

AICD – β-Amyloid Precursor Protein Intracellular Domain

AKT – Protein Kinase B

AMPA – α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid

APH – Anterior Pharynx-defective 1

ApoE – Apolipoprotein E

APP – Amyloid Precursor Protein

ATC – Autophagy-mediated Tau Clearance

AT-N – Aβ, Tau, Neurodegeneration

Aβ – β-Amyloid Peptide

BACE1 – Beta-Site Amyloid Percussor Protein Cleaving Enzyme 1

BACE1-AS – BACE1-Anti Sense

BBB – Blood Brain Barrier

BDNF – Brain-derived Neurotrophic Factor

CAA – Cerebral Amyloid Angiopathy

CD – Cluster of Differentiation

c-DCs1 – Type 1 Myeloid Dendritic Cells

c-DCs2 – Type 2 Myeloid Dendritic Cells

CDK5 – Cyclin-Dependent Kinase 5

circRNA – Circular Ribonucleic Acid

CMA – Chaperone-Mediated Autophagy

CNS – Central Nervous System

CSF – Cerebrospinal Fluid

CVR – Cardiovascular Risk

DCs – Dendritic Cells

DNA – Deoxyribonucleic Acid

E22W42 – $A\beta_{42}$ Peptide with a Mutation in site 22 (Glutamate to Tryptophane)

E22W42-V – E22W42-Sensitized Dendritic Cells Vaccine

ECE – Endothelin Converting Enzyme

EOAD – Early-Onset Alzheimer's Disease

EOFAD – Early-Onset Familial Alzheimer's Disease

FAD – Familiar Alzheimer's Disease

GABA – Gamma-Aminobutyric Acid

GHR – Graft-versus-Host Reaction

GLUT-1 – Glucose Transporter 1

GM-CSF – Granulocyte-Macrophage Colony-Stimulating Factor

GSK-3β – Glycogen Synthase Kinase-3β

HAT – Histone Acetyltransferases

HDAC – Histone Deacetylases

HIC – High-Income Countries

HIV/AIDS – Human Immunodeficiency Virus/Acquired Immunodeficiency Syndrome

HSC – Hematopoietic Stem Cells

IDE – Insulin-Degrading Enzyme

IFN-γ – Interferon Gamma

IL – Interleukin

IRF – Interferon Regulatory Factor

IWG – International Working Group

LC3 – Microtubule-Associated Protein Light Chain 3

LDLR – Low-Density Lipoprotein Receptor

LMIC – Low and Middle-Income Countries

LMPP – Lymphoid Multipotent Progenitors

lncRNA – Long Non-Coding Ribonucleic Acid

LOAD – Late-Onset Alzheimer's Disease

LXR – Liver X Receptor

MALAT-1 – Metastasis Associated Lung Adenocarcinoma Transcript 1

MAPT – Microtubule-Associated Protein

MCI – Mild Cognitive Impairment

mGLUR5 – Metabotropic Glutamate Receptors

MHC – Major Histocompatibility Complex

miRNA – Micro Ribonucleic Acid

mo-DCs – Monocyte-Derived Dendritic Cells

mRNA – Messenger Ribonucleic Acid

ncRNA – Non-Coding Ribonucleic Acid

NEAT1 – Nuclear Paraspeckle Assembly Transcript 1

NEP – Neprilysin

NFTs – Neurofibrillary Tangles

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

NINCDS-ADRDA – National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer's Disease and Related Disorders Association

NMDA – N-methyl-D-aspartate

NMDAR – NMDA Receptor

NVU – Neurovascular Unit

O-GlcNAcylation – O-N-Acetylglucosamine Glycosylation

P22W – Aβ peptide with Mutation in site 22

P24M – Aβ peptide with Mutation in site 24

p75NTR – p75 Neurotrophin Receptor

PBS – Phosphate-Buffered Saline

p-DCs – Plasmacytoid Dendritic Cells

PDM – Aβ peptide with Dutch Mutation

PEN – Presenilin Enhancer

PFDM – Aβ Peptide with Flemish and Dutch Mutation

PFM – Aβ Peptide with Flemish Mutation

PHF – Paired Helical Filaments

PI3K – Phosphoinositide-3 Kinase

PIP3 – Phosphatidylinositol 3,4,5-triphosphate

piRNA – PIWI-Associated Ribonucleic Acid

PP2A – Protein Phosphatase 2A

PrP^c – Cellular Prion Protein

PS1 – Presenilin 1

PS2 – Presenilin 2

PSEN – Presenilin gene

PWT – Wild-Type Aβ peptide

p-τp or p-tau – Hyperphosphorylated Tau

RBP – RNA-Binding Proteins

RISC – RNA-Induced Silencing Complex

RLF4 – Repressor/Activator Protein 1 Localization Factor 4

RNA – Ribonucleic Acid

ROS – Reactive Oxygen Species

SORL1 – Sortilin Related Receptor 1

Th1 – Type 1 Helper T Cells

Th2 – Type 2 Helper T Cells

TLR – Toll-Like Receptor

TNF-α – Tumour Necrosis Factor-α

TREM2 – Triggering Receptor Expressed On Myeloid Cells 2

UDP-GlcNAc – Uridine Diphosphate N-Acetylglucosamine

UPS – Ubiquitin Proteome System

WAR2015 – World Alzheimer Report 2015

ZEB2 – Zinc Finger E-Box Binding Homeobox 2

τp or tau – Tau Protein

INTRODUCTION

Alzheimer's Disease (AD) is a neurodegenerative disease and is the most common cause of dementia. In the United States of America, AD-related deaths have increased 145% in the last 20 years and the cost associated with treatment is estimated to reach 355 billion U.S. Dollars.[1] The number of people with Alzheimer's Disease increases every day, and it is estimated to double or triple in the next 30 years.[2] Due to its complexity, AD research has evolved slowly, with AD treatments only consisting of cholinesterase inhibitors (such as donepezil, rivastigmine, and galantamine) and/or memantine to treat cognitive impairments, which show low rates of amelioration of symptoms.[3]

The involvement and role of Dendritic Cells (DCs) in AD is not completely clear, but these cells are known to localize in the interfaces either between the brain and the blood or the blood and cerebrospinal fluid (CSF). These cells activate the immune system and, consequently, modulate inflammatory responses.[4] Neuroinflammation due to the accumulation of beta-amyloid peptide (Aβ) and cellular responses to AD-related insults is a major component of the pathophysiology of this disease.[5,6] Therefore, due to the antigen-presenting capacity and sentinel properties of DCs, these cells may play an important role in regulating immune responses to fight AD development and onset.[7]

The known functions of DCs along with the success of vaccines using DCs in other pathologies led researchers to investigate the possible efficacy of a vaccine using DCs sensitized with Aβ peptides/oligomers in the treatment of this disease.[8–10] The rationale behind this type of immunotherapy is that DCs can elicit a Th2 response that will induce neutralizing antibodies against cytotoxic Aβ oligomers, concomitantly evoking an anti-inflammatory response. Research focusing on therapeutic alternatives for AD, such as using DCs to develop a vaccine, are extremely important to reach a safer, and more efficient therapy for a disease that affects millions of people worldwide.

I. ALZHEIMER'S DISEASE AND DEMENTIA

1.1. EPIDEMIOLOGY

The National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer Disease and Related Disorders Association (NINCDS-ADRDA) established a list of criteria with the purpose of providing a consistent diagnosis among both clinicians and researchers.[11] The above-mentioned criteria on AD diagnosis allowed epidemiologists to carry more valid and reliable studies concerning Alzheimer's Disease and Dementia (ADD). This section assesses the incidence and prevalence of both disorders.

1.1.1. Incidence

There are 48.6 million people living with dementia, whose cause, in most cases, is AD.[12]

Alzheimer's Disease International (ADI) estimated in their latest epidemiological report – World Alzheimer Report 2015 (WAR 2015) – that the number of new cases per year has most likely reached 9.9 million and the number of total cases worldwide is expected to double every 20 years. According to the cited report, dementia incidence showed an exponential and progressive growth with age. In addition, the increase of incidence of Dementia will be more accentuated in the Americas, Asia, and Africa, and its peak will take place in progressively younger generations, respectively, mainly in Low and Middle Income Countries (LMIC). [2]

In a meta-analysis study carried by GAO *et al.* on the incidence of ADD in three age groups (65 to 74, 75 to 84 and over 85) versus the respective year of birth (1900's to the 1940's), in Western and non-Western countries, the results demonstrated the following:

- × ADD incidence showed an insignificant increase over the years in all age groups in Western countries.
- × ADD incidence increased in non-Western countries in the age group of 65 to 74 years old and remained constant in both the 75 to 84 years old and the above 85 years old age groups.
- × Dementia incidence declined in both Western and non-Western countries for those born in later years.

The decline observed in dementia incidence in people from the same age group but born in later years can be explained by the better economic, sanitary, health and educational conditions, the reduction of cardiovascular risk (CVR), the improved diagnosis' criteria and the availability of proper treatment. Considering that the AD incidence remained statistically constant, and since CVR is a major factor for the development of dementia, it is more likely

that the decline in the dementia incidence is due to the lowering of cerebrovascular disease than AD itself. [12]

1.1.2. Prevalence

Age is a known factor for the instalment of ADD, proven by the exponential increase of prevalence rates after 65 years old.[11]

The AID stated in the above-mentioned report that the prevalence of ADD is higher in Africa and Latin America and lower in Europe. In 2015, there were 47 million people living with Dementia and AD in the world, which represents a prevalence of 5.2% of the population over 60 years old.[2]

The WAR 2015 estimates that the number of people living with ADD in High-Income Countries (HIC) will rise from 19.5 million in 2015 to 42 million in 2050. As well as in LMIC, where the number of cases of ADD is expected to suffer a much more exponential rise from 27 million in 2015 to 89 million in 2050. Therefore, the total of people suffering from ADD will not only increase in all continents but also reach a new global high of 131 million cases in 2050, as seen in **Table 1**.[2]

Table 1: Number of cases and Prevalence of Alzheimer's Disease and Dementia (ADD) in people over 60 years old in Asia, Europe, the Americas, Africa, and the World in 2015, 2030 and 2050. Adapted from the World Alzheimer Report 2015 by Alzheimer's Disease International.[2]

Region	Over 60 Population (millions)	Number of Cases (millions)			Estimated Prevalence (%)		
		2015	2030	2050	2015	2030	2050
Asia	485.83	22.85	38.53	67.18	4.7	7.5	13.2
Europe	176.61	10.46	13.42	18.66	5.9	9.4	16.6
The Americas	147.51	9.44	15.75	29.86	6.4	10.2	18.0
Africa	87.19	4.03	6.99	15.76	4.6	7.3	12.9
World	**897.14**	**46.8**	**74.7**	**131.5**	**5.2**	**8.3**	**14.6**

1.2. PATHOPHYSIOLOGY

Alzheimer's Disease was first described in 1907 by Aloysius Alzheimer, which reported the case of a 51-year-old woman internalized in an asylum. ALZHEIMER stated that the patient's memory was impaired, and episodes of behavioural change started to be more frequent. The author also refers neuronal death and what would later be called

neurofibrillary tangles (NFTs).[13] (for an English translation see STELZMANN, A. *et al.*, 1995.)[14]

This section reviews the pathophysiological mechanisms and events of Alzheimer's Disease and Dementia.

1.2.1. Characterization

Alzheimer's Disease is a neurodegenerative disorder with clinical and physiological signs and symptoms. This disease involves neuropathological events, where amyloid β-peptide (Aβ); tau protein (τp or tau) deposition and Neurofibrillary Tangles (NFTs); and Neurodegeneration are the main hallmark of this disease. The three biomarkers can be referred to as AT-N, where A stands for Aβ, T for τp, And N for degeneration of neuronal cells.[15] These neurological disorders cause progressive cognitive impairment in areas of judgement, logic, memory, and basic functions. Ultimately, ADD leads to death.[16–18]

A correct diagnosis for AD has not always been consensual among experts and a need to harmonize concepts and definitions became mandatory.[11,15,19] In 2011, the International Working Group (IWG) for New Research Criteria for the Diagnosis of Alzheimer's Disease and the National Institute on Aging and Alzheimer's Association (NIA-AA) set a new range of criteria so that a diagnosis and characterization of this disorder could be possible through all stages of the disease, using AT-N as the reference for such.[20] These criteria were later updated and refined in a 2018 Research Framework so that the establishment of a common ground for research and clinical routines is possible. To achieve this universal context of what AD is, the disease was looked at as a continuum rather than segmented phases or units.[15,21] Aβ accumulation is the primary indicator of an AD manifestation or change. However, an AD diagnosis may only be established when there is a concomitant presence of Aβ and Tau proteopathology. Thus, Alzheimer's Disease is defined as a neurodegenerative disease with positive biomarkers for both Aβ deposition and Tau disfunction simultaneously.[15,20]

AD can be simplistically divided in two types and in three subtypes, the latter seen in **Table 2**. Spontaneous AD occurs in individuals that have no presumable hereditary predisposition for AD.[22] The two types of Spontaneous AD can be Late-Onset Alzheimer's Disease (LOAD) and Early-Onset Alzheimer's Disease (EOAD), where the appearance of neurologic and cognitive symptoms sets after and before 65 years of age, respectively. When mutations in specific genes and hereditary influence are evident, the disease is referred to as Familial AD. This concept refers to both Autosomal Dominant Alzheimer's Disease (ADAD) – which is the third type of AD – and some cases of EOAD and very few cases of LOAD.

Consequently, AD types may overlap as their pathogenesis have multiple origins, thus this characterization may present some flaws.[23–26] Therefore, although Spontaneous AD cases are not hereditary, occasionally there's a causal correlation between genetic information, and time of AD-onset neurodegenration.[22]

Table 2: Types of Alzheimer's Disease according to the influence of hereditary factors and the time of onset and diagnosis of the disease. The highest prevalence is seen in LOAD and EOAD, where cases are mostly spontaneous. Concerning these two types, only 5% of cases are Familial EOAD; and only an infimal percentage of LOAD cases fall under the Familial AD definition and diagnosis. Under 2% of cases are ADAD. The age of onset of ADAD can vary according to multiple factors.[23–26]

	Age of Onset of Symptoms	Prevalence
LOAD	≥ 65 years old	82% - 92%
EOAD	< 65 years old	6% -16%
ADAD	Any age	< 2%

1.2.2. Genetic and Epigenetic Involvement

The role of genetics in Alzheimer's Disease is extremely extensive and new knowledge of its role comes up every year.[17] Whether it is an hereditary component, an environment-related mutation or age-induced alterations, this connection is ever present in both Preclinical and Clinical phases and in all types of AD. However, the genes and proteins involved in pathogenesis and time of disease onset may vary among the three types of AD.

A. APP, PSEN1 and PSEN2

In Early-Onset Familial (FAD) AD (EOFAD) patients, mutations on *APP* gene, *PSEN1*, and *PSEN2* were found. These genes mediate the production of β-Amyloid Precursor Protein (APP), Presenilin 1(PS1) and Presenilin 2 (PS2), respectively. APP gets cleaved and results in Aβ, and PS1 and PS2 are involved in the cleavage of APP by making up γ-secretases. This discovery correlated well with AD pathogenesis in individuals with this particular type of AD. For that reason, it is accepted that these mutations are major risk factors for the development and early-onset of AD.[17,22,26]

B. BACE1

Mutations in the Beta-Site Amyloid Percussor Protein Cleaving Enzyme 1 (BACE1) gene that correlate to AD have not been reported. However, some epigenetic events, like methylation, acetylation and microRNA can regulate the expression of BACE1. Therefore, despite not having a genetic involvement in AD, DNA and RNA processes are involved in

BACE1 gene regulation and protein expression, which, ultimately, impacts AD development and onset.[27]

C. ApoE ε4

Apolipoprotein E (ApoE) is a protein responsible for lipid transport., and it can assume different isoforms: ApoE3 is the most common isoform; ApoE2 and ApoE4 are rarely present in disease-free patients. These two infrequent isoforms vary from the ApoE3 isoform by a change in a single amino acid – ApoE4 contains an arginine in position 112 (instead of a cysteine) and ApoE2 contains a cysteine in residue 158 (instead of an arginine). These changes interfere with the protein's solute affinity and binding rates. The presence of ApoE4 increases the risk of developing AD by 4.4.[28,29]

EOAD patients showed higher homozygotic ApoE ε4 allele frequency and more severe forms of AD when compared to LOAD patients.[26] Although alterations in this gene may be present in all AD types, it is in LOAD that it constitutes the major genetic risk factor for AD development.[17,30]

No causal effect was established between this gene and cognitive impairment. ApoE4 is known to have a negative effect on the Aβ accumulation process, which, in turn, has a negative impact on disease severity and progression but a non-relevant effect on cognitive function.[23,24,26,31]

D. *SORL1*

Sortilin Related Receptor 1 (*SORL1*) gene expression plays a very important role in Alzheimer's Disease. SORL1 is capable of sorting neurons for APP, and regulates its course, either driving it to the retromer recycling or to the late endosomal pathways.[22,32,33]

There is a wide set of variants of this gene present in heterogeneous populations that modify the expression of the SORL1 transmembrane neuronal protein. An under-expression of SORL1 determines a rise in Aβ production, therefore increasing the risk of developing AD.[32,33]

E. TREM2

TREM2 is a gene highly expressed in white matter and abundantly in the hippocampus and neocortex. This gene regulates two important reactive pathways: one regulates phagocytosis in microglia, which plays a protective role; and the other controls cytokine production and secretion, supressing inflammatory processes and inducing the activation of survival and repairing mechanisms. Mutations that result in loss of function of this gene have been related

to ADAD and EOAD, with the R47H variant presenting the strongest association with AD ($p<0,001$).[22,30] These mutations result in Aβ clearance deficiency and systemic inflammatory responses, which lead to Aβ deposition and neuronal death, respectively.[22,30]

F. Methylation

Methylation is a very common epigenetic modification performed by a specific enzyme called DNA Methyltransferase. In mammals, this process consists in the addition of a methyl group to the Carbon-5 of a cytosine nucleotide in DNA, usually at CpG locations present in gene promoters. As this epigenetic process occurs, the expression of the downstream gene is not executed, and consequently the respective protein is not produced.[22,34]

Immunoreactive assessments proved that a significant hypomethylation in neurons of the entorhinal cortex (a highly vulnerable region in AD) exists in AD patients.[34]

TOHGI, H. *et al.* and FUSO, A. *et al.* found much lower methylation rates of the *APP* and *PS1* gene promoters in older individuals, free of AD.[35,36] The hypomethylation of these regions can result in an increased APP production and processing, as an hypomethylated promoter cannot prevent gene expression accordingly. Consequently, this can result in a higher rate of Aβ production and deposition. Aβ is a trigger for epigenetic malfunctions, as it was found that this peptide is able to induce global hypomethylation.[22] Therefore, this can constitute a never-ending cycle, as seen in **Figure 1**.

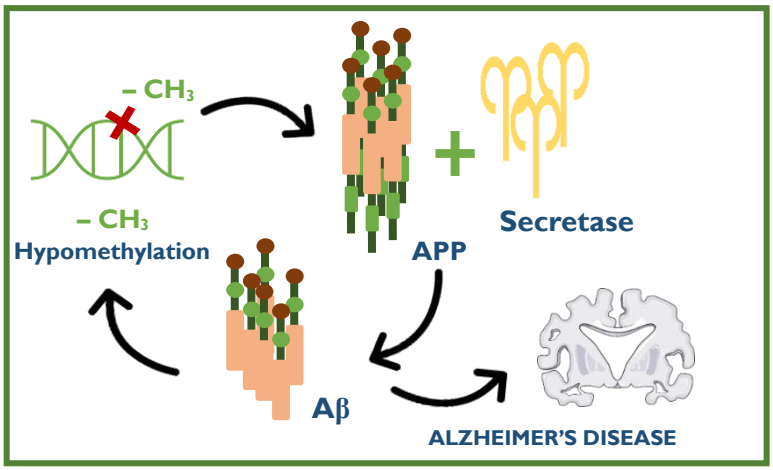

Figure 1: Hypomethylation promotes downstream expression of gene.[34] In older people, this event is widely present, thus increasing quantities of APP and γ-secretases, consequently increasing Aβ formation.[35,36] With an increase in Aβ formation and deposition, not only does it induce hypomethylation but also rises predisposition for AD.[18,22] Therefore, the lack of CpG methylation in the *APP* and *PSEN1* promoters is a risk factor for AD.[22,35,36]

G. ncRNAs

Following the process of transcription, 98% of RNA is not translated into proteins. These RNA strands are called non-coding RNAs (ncRNAs).[22,37] Despite not being translated, they play a very important role in chromatin and nuclear structure, and in epigenetic regulation.[22] ncRNAs can bind DNA, RNA, and proteins, which enables them to regulate various processes like gene transcription, mRNA translation, RNA turnover, and synaptic plasticity.[37,38] ncRNAs can be comprised as small RNAs (sRNAs) and long non-coding RNAs (lncRNAs), according to their size. sRNAs can be subdivided into micro RNAs (miRNAs), circular RNAs (circRNAs), short interfering RNAs (siRNAs), and PIWI-associated RNAs (piRNAs). lncRNAs are divided and categorized according to their localization.[22,37]

lncRNAs contain more than 200 nucleotides and do not have an appreciated open reading frame.[22,39] However, they possess relevant physiological functions, such as: stabilizing, repressing and activating gene expression[39]; interacting with chromatin, DNA, RNA and RNA-Binding Proteins (RBP)[38]; and containing structural parts of mRNA[39]. In AD, the lncRNA BACE1-antisense (BACE1-AS) is highly expressed and competes with other regulating factors for the BACE1 mRNA. This leads to upregulation of BACE1, which increases expression of this protein, leads to Aβ accumulation, and AD development. Other lncRNAs can also have an impact on AD, like MALAT1 and NEAT1 that regulate synaptic function and BC200 that is present in specific areas of AD-affected brains.[22,39]

miRNAs are made up of approximately 22 nucleotides that inhibit protein translation by causing mRNA degradation via the RNA-Induced Silencing Complex (RISC). This process is involved in many cellular mechanisms, such as differentiation, proliferation, and apoptosis. These RNAs are extremely and evolutionarily conserved. This RNA type is found mostly in the Central Nervous System (CNS), with a vast quantity located in presynaptic and postsynaptic compartments. Several types of miRNAs are linked to dendritic spinal damage, BACE1 modulation and/or tau hyperphosphorylation.[22,37,40]

circRNAs are believed to be the product of splicing of precursor RNAs. The 5' end of a preceding exon is covalently bound to the 3' end of a downstream exon, which means that these ends are not free; and are consequently resistant to exonucleases. These sRNAs modulate miRNAs' effect on mRNA stability and expression. Additionally, circRNAs can interact with splicing factors and RBP, and can be translated to peptides (despite being ncRNAs).[37,38]

piRNAs are about the same size as miRNAs but appear much more frequently. These ncRNAs require a RBP of the PIWI family to regulate gene expression. They do this through transposon silencing, gene transcription, mRNA turnover and translation, and interaction with other ncRNAs. These RNA type has been identified in AD patients, and some piRNAs have been proposed as markers. Oxidative stress and cellular homeostasis failure show correlation with piR-38240 and memory impairment with aca-piR-4 and aca-piR-15.[37,38,41]

H. Chromatin Remodelling

In humans, histone proteins combine themselves with DNA to form chromatin, which is the compact packaged state of DNA. Histones are octamers constituted by four cooperative proteins: H2A, H2B, H3, and H4. These proteins can be exposed to epigenetic alterations like acetylation, methylation, phosphorylation, ubiquitination, and others, which can result in several events, such as: chromatin arrangement; limiting or enhancing accessibility of DNA strands to various enzymes; and activation or repression of several genes.[22] The most studied and well-understood epigenetic process involving histones and chromatin in AD is acetylation. The two families of enzymes that are the most responsible for this process are histone deacetylases (HDAC) and histone acetyltransferases (HAT).[22,42] In a mouse model of AD, the brains of mice showed reduced histone acetylation levels when compared to normal individuals, which result in memory impairment and loss of synaptic plasticity.[42,43]

1.2.3. Neurodegenerative Pathology

Alzheimer's Disease consists of two different stages – Preclinical AD and Clinical AD[15]. In the first stage, there are two phases – Biochemical Phase and Cellular Phase – which involve genetic alterations, protein disfunction, and cell and tissue damage.[44] The second stage overlaps with the third phase – the Clinical Phase – which denotes to the observable symptoms and signs of the disease.[15,44] These phases do not occur sequentially, as they evolve simultaneously and interdependently.

The types of lesions and alterations in AD can be classified as follows: positive lesions, where an accumulative process occurs; negative lesions, where a loss of either physiological function or cell masses occurs; and reactive processes, where inflammation and synaptic plasticity play the major role.[45]

1.2.3.1. *Phase I: Biochemical Phase*

Aβ and Amyloid Deposits

The β-amyloid peptide accumulation is a positive lesion, and is the major biomarker of AD. It was characterized by GLENNER and WONG (1984) and first identified as the main constituent of the meningovascular amyloid on AD patients affected by Cerebral Amyloid Angiopathy (CAA) in 1984 and as the main constituent in neuritic plaques in 1985 by MASTERS *et al.*[46,47]

A. Aβ Composition and Formation

Aβ is formed in endosomes and is the result of the amyloidogenic pathway of APP processing.[23,48] This pathway involves the cleavage of APP by β-secretase enzyme (BACE1) at its N terminus, forming two products, which are APP-β and C99. The γ-Secretase Complex (γ-secretase, for short) – composed of presenilin (PSEN), presenilin enhancer (PEN), APH, and Nicastrin – is then responsible for the cleavage of C99, releasing the C terminus Aβ. The remaining Aβ C-terminal end is posteriorly processed by the same complex producing 43 to 51 amino acid-long peptides (cleaved further to 40 and 42 amino acid-long Aβ peptides) until the γ-secretase releases the Aβ peptide by presynaptic and postsynaptic modulation.[23]

Aβ exists in very different assembly states, usually following a maturation process with low weight oligomers, such as monomers, dimers, trimers, and tetramers; and higher-orders oligomers and β-sheet conformations, such as protofibrils and mature fibrils. Protofibrils and fibrils are insoluble conformations, which makes them the major components of senile plaques. However, soluble Aβ assemblies are capable of spreading through the brain, which makes these Aβ oligomers the most potent to cause synaptic and neural impairment and degeneration.[48,49]

B. Aβ Receptors

Aβ, and especially soluble Aβ oligomers, showed the capacity to bind with different receptors. This Aβ-Receptor interactions result in cellular dysfunction and oxidative stress in neurons, which lead to neuronal toxicity.[48]

An experiment conducted by SHANKAR *et al.* showed that Aβ oligomers can bind with N-methyl-D-aspartic Acid (NMDA) Receptor (NMDAR) post-synaptically, activating it. Consequently, a dysfunctional increase in calcium influx occurs, leading to neuronal changes such as loss of dendritic spines and synaptic plasticity and function.[48,50]

PERINI *et al.* studied the interaction between Aβ and the p75 Neurotrophin Receptor (p75NTR). Aβ and p75NTR interact via the receptor's extracellular domain, inducing activation of caspases, production of Reactive Oxygen Species (ROS), and cellular oxidative stress.[48,51] Aβ interacts with other cytokines in a synergistic way, therefore enhancing the neurotoxic properties of the Aβ-p75NTR interaction. Aβ-bound p75NTR and cytokines trigger apoptotic pathways, mainly in the hippocampus.[48,51]

Aβ also binds with high affinity to the Cellular Prion Protein (PrP^c), a protein that can suffer conformational changes to a pathological and infectious state that is involved in neurodegenerative processes. Aβ and PrP^c show, then, affinity to the Metabotropic Glutamate Receptors (mGluR5) that act as co-receptors for these two proteins. These two receptors act in astrocyte upregulation of Aβ, and play a catalysing role for neurotoxic events, such as Ca^{2+} release and stimulation of APP translation.[48]

C. Aβ Degradation and Clearance

Aβ accumulation is not only related to an excessive production of APP and Aβ, but also due to mechanism deficiencies of Aβ clearance. Proteases play the major role in Aβ degradation, where Neprilysin (NEP), Endothelin Converting Enzyme (ECE), Insulin-Degrading Enzyme (IDE), and Plasmin are the main effectors in AD[48].

Neprilysin and ECE are zinc metalloproteinases, which active site binds to Aβ, located in the extracellular space.[48,52] IWATA *et al.* demonstrated that the extracellular space is the main location of Aβ metabolism. IWATA *et al.* and ECKMAN *et al.* demonstrated that NEP and ECE are involved in degradation of Aβ, respectively. IWATA *et al.* still reported that the effects of catabolism of Aβ decrease with age, and that catabolic mechanisms can have a bigger role in AD pathology than anabolic ones.[48,52,53]

Insulin-degrading Enzyme is also a zinc metallopeptidase and is responsible for Aβ degradation in two occasions. Firstly, it degrades the β-Amyloid Precursor Protein Intracellular Domain (AICD), the cytoplasmatic component of APP, which has transcriptional implications in APP regulation.[48,54] Secondly, it shows specific linkage to substrates with a β-sheet conformation. IDE is the most crucial Aβ clearance agent in hippocampal lysates and has the capacity of degrading Aβ both in cytoplasm and cerebrospinal fluid. Action of IDE lowers with age, which supports the age-associated impairments in AD.[55]

Aβ transport through the blood brain barrier (BBB) and clearance by astrocytes and microglia is also a key factor in clearance and regulation of Aβ. The main agent involved in

this mechanism of Aβ clearance is ApoE. This lipoprotein is secreted by microglia and astrocytes into the interstitial fluid of the brain, where it can bind both soluble and fibrillar Aβ and consequently mediate its clearance via the BBB, degrading proteases, and uptake by astrocytes and microglia. ApoE4, an isoform of ApoE, is the less efficient in transporting Aβ, which makes its carriers much more susceptible to AD development.[48,56]

D. Aβ Deposition

Aβ peptides aggregate into β-sheet conformations, as previously mentioned, with $A\beta_{42}$ showing a greater amyloidogenic capacity.[23] The aggregation process of the β-amyloid peptide can occur in: a metal-dependent aggregation, which forms ionically bridged aggregates, covalently crosslinked oligomers, and seeds for soluble oligomers and amyloid fibrils.[48] These seeds work as templates for the formation of larger aggregates.[23] Soluble oligomers and Aβ fibrils constitute the non-metal dependent Aβ aggregation type.[48]

Extracellular deposits can be revealed with anti-Aβ antibodies for Aβ-specific plaques or with Congo Red or Thioflavin S staining for the presence of amyloid or β-sheet conformational structures.[45]

There are three types of Aβ extracellular deposits: diffuse, neuritic, and compact plaques.[57]

Diffuse plaques are amorphous plaques that can range from 10 μm to 20 μm and have ill-defined contours. These plaques do not have dystrophic neurites, are not associated with glial responses, and do not cause synaptic dysfunctions. However, they can bind with ApoE in an initial aggregation stage. They have also been associated with individuals whose cognitive status is reported as normal, therefore not being sufficient to establish an AD diagnosis. They react positively with silver staining, but negatively with Congo Red or Thioflavin.[45,57]

Neuritic plaques can be subdivided in two different categories: primitive and focal neuritic plaques. Primitive plaques are oval structures ranging from 20 μm to 60 μm in diameter and contain mainly $A\beta_{40}$ and neuritic alterations. However, they show no staining with Congo Red or Thioflavin S since the Aβ is not in a β-sheet conformation. They are associated with astroglial responses. Focal neuritic plaques are the same size as primitive plaques, but have a core majorly constituted by $A\beta_{42}$ encircled by fibrillar Aβ deposits. The surrounding area of the core, called corona, has NFT-associated neuritic dystrophies, astrocytic components, and activated microglia. These plaques are revealed by Congo Red and Thioflavin S. Focal

plaques cause synaptic damage, neuronal loss, and cognitive impairment, which makes them a valid basis for an AD diagnosis.[45,57]

Compact plaques are 5 μm to 15 μm in diameter with a dense core that has no neuritic corona.[57]

E. Aβ Pathology

Aβ pathology can be characterized by three different aspects: topography of Aβ plaques distribution (Aβ phase and CAA-affected vessels); quantitative measures of Aβ in a specific brain location (Aβ loads and soluble Aβ levels); and qualitative changes of Aβ species (such as $Aβ_{40/42}$, $Aβ_{N3pE}$, and $Aβ_{pSer8}$).[58]

Aβ pathology can present three different aspects:

1) different topographical distributions, which depends on the stage of the disease. There are two ways to topographically define AD-related Aβ pathology. According to THAL *et al.*, there are five phases of Aβ deposition, in which brain regions are hierarchically involved. In phase 1, the only brain region presenting Aβ deposition is the neocortex. In phase 2, additionally to the neocortex, the entorhinal region is also affected. In phase 3, Aβ starts to appear in several subcortical regions, such as the amygdala, the thalamus, the hypothalamus, among others. In phase 4, the red nucleus is also widely affected, as well as the substantia nigra. In phase 5, β-Amyloidosis has reached several other areas like the reticular formation of the pons, the pontine nuclei, the cerebellar molecular layer, the dorsal and reticulo tegmental nuclei, among many other brain regions. Phases 1, 2 and 3 correspond to Preclinical AD, and phases 4 and 5 correspond to Clinical AD.[59–61] BRAAK *et al.* considered three different stages for Aβ deposition. In stage A, amyloid deposits occur in basal portions of the cortex. In stage B, the isocortex is completely involved, excluding the primary cortices, and the hippocampus is mildly affected. At stage C, the Aβ deposits can be found in all isocortex areas, including sensory and motor core fields.[45]

2) different quantities of Aβ species, such as Aβ loads and soluble, dispersed, membrane-associated, and plaque-associated Aβ levels.[58,60]

3) various qualitative parameters like differences in Aβ species, whether in amino acid residue quantity ($Aβ_{40}$ and $Aβ_{42}$, the most present in AD), in conformational status (monomers, oligomers, protofibrils and fibrils), or in post-translational biochemical properties (Aβ truncated at the two first amino acids of the peptide's N-terminal, followed by pyroglutamate formation – $Aβ_{N3pE}$ – that enhances aggregation processes by changing

biophysical properties of Aβ fibrils; and Aβ phosphorylated in the Serine 8 – $A\beta_{pSer8}$ – that promotes oligomerization of Aβ, which serves as nucleation sites for fibril formation).[58,60]

A study carried in three cohorts by THAL *et al.* demonstrated the following: all these aspects correlated well with each other and with AD neuropathological events non related to Aβ; the post-transcriptional changes and topography of Aβ plaques also show correlation with the increase of cognitive impairment (even if MCI and dementia correlate better with NFTs); and the three aforementioned parameters link with NFTs and Aβ plaque pathology.[58]

Tau protein and Neurofibrillary Tangles

Another positive lesion of AD is the intracellular formation and accumulation of neuropil threads and mostly neurofibrillary tangles (NFTs).[45] They were first discovered by Alois Alzheimer in his case study of a 51-year-old woman.[13,14]

NFTs and neuropil threads are made up of aggregated insoluble tau protein that folds into β-sheet conformations. These aggregates are either paired helical filaments (PHFs) or twisted ribbon-like assemblies, and consist of hyperphosphorylated misfolded tau (p-τp or p-tau).[18,23,62] τp is a microtubule-associated protein (MAPT) responsible for stabilization of the microarchitecture of neuronal cells by polymerizing tubulin into microtubules.[18,63]

A. Tau Hyperphosphorylation

The phosphorylation status of tau protein (τp or tau) can be mediated by kinases and phosphatases. The two main kinases that phosphorylate τp are Glycogen Synthase Kinase-3β (GSK-3β) and Cyclin-Dependent Kinase 5 (CDK5).[63] GSK-3β phosphorylates tau via the PI3K/AKT/GSK-3β pathway. This pathway starts with increased levels of Phosphatidylinositol 3,4,5-triphosphate (PIP3), which is the major second messenger and activator of Phosphoinositide-3 Kinase (PI3K). Consequently, overactivated PI3K catalyses phosphorylation and activation of AKT, which in its turn hyperphosphorylates GSK-3β. This results in hyperphosphorylation of τp by GSK-3β due to PI3K/AKT signalling pathway dysfunctions.[64] CDK5 induces p-τp via PHF formation. PHF forms due to CDK5 activators p35 and p39. This process starts with neuronal insults and toxic factors that increase calcium influx and promote cleavage of the two activators by calpain, resulting in hyperactive complexes consisting of CDK5 and p25 and p29 (resulting fragments of the cleavage of the two activators p35 and p39, respectively). This hyperactive state of CDK5 induces tau hyperphosphorylation and formation of oligomers and paired helical fragments.[63] On the other hand, Protein Phosphatase 2A (PP2A) is responsible for taking phosphate groups off of

tau, therefore playing an important role in preventing the formation of p-τp and its misfolding into NFTs. These kinases and phosphatases do not work independently: CDK5 regulates phosphorylation of GSK-3β by activation of AKT; PP2A can deactivate GSK-3β, impeding it from phosphorylating tau; and overactivated GSK-3β can inhibit PP2A by methylation. In conclusion, dysregulation of pathways of these enzymes (either independently or collaboratively) can lead to p-τp, aggregation and formation of NFTs.[63,64]

Inflammation is a reactive process that also plays a key role in AD and in tau-related positive lesions. This process is extensively related with cytokines, such as IL-β, IL-6, IL-8, IL-18, and TNF-α, and microglia. IL-β and IL-18, for example, cause an increase in p-τp and mediate tau phosphorylation through NMDAR regulation, respectively.[63] Microglial inflammation processes will be reviewed in the cellular phase section.

Besides phosphorylation, Tau can also suffer other post-translational modifications such as acetylation, proteolytic cleavage, and glycosylation.[63] Acetylation of tau in certain residues, like K280, can be a primary step of its accumulation since it is more prone to aggregation.[63,65,66] On the other hand, not only does elevation in deacetylation by Histone Deacetylase 6 (HDAC6) of tau prompt it to hyperphosphorylation, but acetylation by p300 inhibits its aggregation.[63,65,66] Tau also has acetyltransferase activity, which means it can auto-acetylate itself. Therefore, this implies that impairments in this balance between acetylation and deacetylation processes may play a role in regulating tau aggregation.[63] The truncation of tau by overly activated caspase, calpain, and AEP (a lysosomal cysteine proteinase), as found in AD patients' brains, can result in seeding for τp accumulation.[63] N-glycosylation of tau increases formation of p-tau, and O-N-Acetylglucosamine glycosylation (O-GlcNAcylation) of tau decreases and suppresses its aggregation. A study using 3xTg-AD mice conducted by GATTA, E. *et al.* demonstrated that O-GlcNAcylation is decreased in the hippocampus of these models, which correlates with tau aggregation and synaptic dysfunction.[63,67] Importantly, glucose is converted into UDP-GlcNAc, which is involved in these reactions. Therefore, an impairment in glucose uptake in the brain could contribute to the defect in O-GlcNAcylation, and ultimately lead to an increase in p-tau, its accumulation, and neuronal impairments, such as neurodegeneration.[63] This glucose-related mechanisms impairment in the brain lead STEEN, E. *et al.* to go as far as to call AD type-3 diabetes.[68]

B. Tau Clearance and Degradation

The clearance of tau can follow two distinct pathways: the ubiquitin-proteasome system (UPS) or autophagy-mediated tau clearance (ATC).

UPS is responsible for the regulation of protein levels in the cell and elimination and clearance of misfolded and damaged proteins. This process happens by binding ubiquitin with a substrate (a misfolded or damaged protein, usually) that delivers it to the proteasome, which will proceed to degrade this substrate. Tau co-immunoprecipitates with proteasome subunits and binds with its core particle, which leads to the decline of the UPS activity. In AD, high levels of ubiquitin and a presence of an aberrant ubiquitin mutation – that is resistant to ubiquitination and inhibits proteasomes – were reported in NFTs. Therefore, dysfunctions in the UPS are related to tau aggregation and neurodegeneration in AD.[63,69]

Autophagy consists of catabolic processes that lead to degradation of cytoplasmic components. Autophagy clears long-lived proteins and even damaged organelles. There are three types of autophagic processes: macroautophagy (the most studied); microautophagy; and chaperone-mediated autophagy (CMA). Several studies reported that inhibiting autophagy-lysosome pathways lead to an impairment in tau degradation, while inducing this system facilitates τp degradation and showed protectiveness against its toxicity. However, CMA produces amyloidogenic tau fragments that enhance tau aggregation.[69]

Regarding the clearance mechanisms of τp, UPS plays a more pivotal role with monomeric tau, whereas autophagy is the main responsible for the degradation of tau oligomers and aggregates.[63]

C. Tau Aggregation

Tau aggregation starts with the oligomerization of tau monomers into fibrils, filaments and NFTs. There are several factors that impact the aggregation of τp, among which are post-translational mechanisms, such as phosphorylation, acetylation and glycosylation; and pathological seeds of tau protein.[70]

Once τp aggregates into fibrils, it is proven to show a prion-like ability, which allows it to spread transsynaptically to other brain regions, and consequently induce further seeding and aggregation.

Tau accumulation occurs differently according to the neuronal domains: tangles relate to τp aggregation in the soma; neuropil threads appear in the dendrites; and the corona of neuritic plaques is associated with axonal processes, which are rich in τp.[45]

D. Tau and Aβ

The relationship between Tau and Aβ remains very unclear, despite being the two main biomarkers of AD.[63]

Aβ can trigger Tau pathology by enhancing hyperphosphorylation of τp in mice, exacerbating NFTs formation, and accelerating the propagation of tau through the brain. Together, these mechanisms facilitate cognitive impairment and dementia, characteristic of tau pathology.[23,63] However, Tau can mediate Aβ-related toxic mechanisms by allowing Aβ to activate GSK-3β, and can even be mandatory for synaptotoxicity and cognitive impairment involved in the Aβ pathway.[63,71]

E. Tau pathology

The neurotoxicity of tau is assumed to be caused by NFTs. However, recent studies suggested that neurodegenerative mechanisms and symptoms might appear before NFTs establishment, probably because of neurotoxicity due to soluble pathological τp. It is likely that hyperphosphorylated tau oligomers induce mitochondrial abnormalities, oxidative stress, synapse loss, leading to neuronal damage and cognitive loss.[63]

Tau pathology, as does Aβ, progresses to and affects different brain regions at different stages. BRAAK *et al.* defines six stages for AD-related neurofibrillary lesions. In stage I, lesions occur in the transentorhinal region and extend to the entorhinal region in stage II. In stage III, not only do the lesions in stage I and II get more severe, but also reach the neocortex. In stage IV, the areas affected in previous stages are more severely impacted but suffer very small changes in following stages. In this stage, the lesions expand to the mature neocortex, particularly in the middle temporal convolution. In stage V, lesions extend to the first temporal convolution and areas of the frontal, parietal, and occipital neocortex (peristriate region), and secondary locations of the neocortex start to develop a similar lesion pattern (in a less pronounced way). Finally, in stage VI, the lesions reach the secondary and primary neocortical regions and extend to the striate area.[72,73]

ApoE

Apolipoprotein E results of the expression of the *APOE* gene, which presents three polymorphic alleles – ε2, ε3, and ε4 – that generate ApoE2, ApoE3, and ApoE4, respectively. The difference between these isoforms lies on the amino acid residues 112 and 158: ApoE2 has cysteine in both residues; ApoE3 has a cysteine in residue 112 and an arginine in residue 158; ApoE4 has an arginine in both residues.[74] APOE acts as a lipid homeostasis regulator

through lipid transport, more specially cholesterol transport. In the central nervous system, apolipoprotein E is produced majorly by astrocytes and, to a lesser extent, by microglia. ApoE binds cholesterol and other lipids through the carboxy-terminal and redistributes it to neurons by binding to low-density lipoproteins (LDL) receptors (LDLR) through the amino-terminal domain.[23,56,75]

A. ApoE Isoforms in AD

In AD patients, ε4 allele frequency is approximately 20% higher, compared to healthy people. In individuals who express this allele, the proneness of developing EOAD or LOAD increases markedly.[23,75] ApoE4 carriers are also prone to atherosclerosis, vascular disease and type 2 Diabetes, which are, by themselves, risk factors for AD. Therefore, patients with one or more of the diseases aforementioned who are ε4 carriers are at a higher risk of developing AD.[75]

As mentioned before, the ApoE4 is the strongest genetic risk factor for AD. On the other hand, the ε2 allele seems to be protective against AD.[76] However, the presence of the ε4 gene outweighs the presence of ε2, which means the protective action of ε2 is not absolute.[56]

B. ApoE and other AD-related Pathologies

ApoE is also relevant in ADD because of its major isoform-dependent interaction with Aβ. Aβ burdens, plaque deposition, neurotoxic Aβ species, and CAA severity are higher in ε4 carriers, followed by ε3 and ε2 carriers. ApoE and its isoforms show a modulating role in Aβ metabolism.[56,75]

ApoE can impact Aβ metabolism in several stages: in production, ApoE4 intervenes in APP transcription and amyloidogenic processes, increases peptide seeding, and promotes fibril formation; in aggregation, ApoE binds to Aβ and forms massive co-aggregates that promote the formation of amyloid plaques; in clearance, ApoE impairs the diverse mechanisms involved in this stage, by damaging the blood brain barrier integrity, binding to cellular Aβ receptors, and inefficiently degrading Aβ peptides (markedly in ApoE4 individuals).[23,56,77]

ApoE also plays a role in tau pathology. ApoE4 homozygotic individuals show higher p-tau levels and more tau aggregates, when Aβ pathology is present.[56,77]

Additionally, ApoE4 also causes: neuronal degeneration by having both a strong pro-inflammatory and a weak anti-inflammatory function; dysfunction of dendritic plasticity and synapses through reduction of synaptic proteins and LDL, AMPA, and NMDA receptors; and lipid metabolism impairments due to inefficiency for neuronal delivery of cholesterol.[56,75]

1.2.3.2. *Phase 2: Cellular Phase*

The resulting damages in the biochemical phase evolve and start eliciting responses that go from reversible proteopathic reactions to irreversible compensating mechanisms that impair the brain's homeostasis, which can occur in a cell or non-cell autonomous pathway – the cellular phase.[44]

The second phase starts before the biochemical phase reaches its peak, such as amyloid plaques and NFTs. Therefore, the two phases can occur synchronically, but the cellular phase comes after the biochemical one.

A. *Neurovascular Unit*

The neurovascular unit (NVU) is an anatomical and functional unity that regulates the cerebral blood flow. It describes a close relationship between the cellular components of the brain (neurons, interneurons, astrocytes) and the blood vessels (endothelial cells, pericytes, and myocytes).[44,78]

The neurovascular unit is intimately related to the BBB, which are both compromised in Alzheimer's Disease. Aβ, ApoE4, GLUT1, hypoxia, atherosclerosis, and traumatic brain injuries cause degenerative responses of brain vasculature components, which lead to the breakdown of the BBB. Following this, Aβ and τp clearance and metabolism impairments appear, and cause Aβ deposition, which leads to necrosis, microaneurysms, and CAA.[44,78]

B. *Neurons*

Neuronal and synaptic plasticity disfunctions are a major hallmark of AD, since they correlate best with cognitive impairment..[49]

Aβ production can be modulated by neuronal electrical activity through the β-secretase site in APP. On the other hand, increased Aβ levels, particularly $A\beta_{42}$, lead to depression of synaptic transmission.[79] Synaptic disfunction due to Aβ appears before plaque formation, which indicates that the Aβ oligomers are the ones responsible for neuronal degeneration. Aβ impairs glutamate uptake, which leads to its accumulation in the extrasynaptic space, causing excitotoxicity and neuronal death. Healthy neurons can also respond to Aβ

increased levels, which suggests that synaptic disfunction can also occur in a non-cell autonomous way.[49,71,79]

Tau protein can also induce synaptic disfunction, both directly (by interacting with several proteins, such as PP2A and Nectin-3, involved in cell adhesion and synaptic plasticity) and indirectly through Aβ-induced mechanisms (p-tau colocalizes with Aβ in synaptic terminals and both cause synapse numbers reduction). The synaptic disfunctions caused by τp are not due to NFTs, but soluble tau species.[71]

C. Astrocytes

Astrocytes are deeply involved in synapse transmission, brain vasculature and BBB integrity and function.

The astroglia population, along with oligodendrocytes, synthesise all brain cholesterol and secrete Apolipoprotein E and J. The astrocyte-derived cholesterol and the brain lipid metabolism are crucial for both synaptogenesis and clearance of Aβ over the BBB. Astrocytes have many different receptors and machinery relevant to monitor neuronal function (glutamate, GABA, and acetylcholine receptors; communication with the BBB; and Aβ and τp clearance), thus possessing an extremely vital role in synaptic formation, and brain function regulation (induction and elimination of synapses; long-term potentiation; short-term plasticity; and Ca^{2+}, neurotransmitters, and modulators homeostasis).[44]

The mentioned events are normal responses, but there can also be reactive responses, such as astrogliosis, which consists in astrocytic morphological changes, expression of pro-inflammatory factors, and excitotoxic processes. In AD, astrogliosis interferes with lymphatic flow, thus contributing to clearance deficiency of Aβ.

In astrocytes there can also be negative lesions, with astrocytic atrophy and increased excitability. This causes metabolic and morphological disturbances, which in turn lead to synaptic disfunction.

D. Microglia

Microglia are the phagocytic cells of the CNS and interact with neurons through their ramified processes. The majorly expressed receptor in microglia is TREM2, which mediates phagocytic function and anti-inflammatory cytokines expression. In AD, mutations in the TREM2 gene increase the risk of LOAD, since the underexpression of this receptor hinders phagocytosis of Aβ plaques, thus impairing Aβ clearance.

Microglia also contribute to inflammatory processes by expressing other receptors (such as CD36 and TLR-4) that activate innate immune responses in response to Aβ aggregates. This inflammatory processes are extremely complex, because they can either be beneficial or damaging.[44]

1.2.3.3. *Phase 3: Clinical Phase*

Clinical AD syndromes are complex and heterogeneous among patients. However, it is possible to distinguish two different syndromes, according to the cognitive function level and accompanying functional profile: Mild Cognitive Impairment (MCI) and Dementia.

A. Early stages and Mild Cognitive Impairment

In early stages of the disease, some symptoms start to appear, such as anxiety, changes in mood and sleep, depression, and apathy. However, they are not specific to AD and are not sufficient to establish a diagnosis.[15,80]

MCI happens in Preclinical AD and can be described as a patient-specific behavioural syndrome, where cognitive detriments like memory failure and disorientation are noticeable but do not affect a person's daily actions and functions.[81]

B. Dementia

Dementia is the consequent occurrence of AD-related disfunctions and can be divided in three stages: mild, moderate, or severe dementia.

In mild dementia, individuals can present memory impairments, but also aphasia and visuoconstructional problems. In this stage, recent declarative memory is much more affected than short-term and old declarative memory. Depressive episodes and emotional disturbances are frequent in mild dementia. The patient is still independent, but needs support and help to do more complicated tasks.[82]

In the moderate stage of dementia, recent memory loss is more significant, logical thinking starts deteriorating noticeably, and language impediments become more apparent. In this stage, patients show problems with recognizing people's faces, incontinence, disorientation, and hallucinations. The emotional balance is greatly compromised in the second stage of dementia, where extreme anosognosia, loss of emotional control and even aggressive behaviours start to be more present. Patients need to be closely supervised due to the inability to perform simples tasks and putting themselves (and their caregivers) in danger.[82]

By the time patients reach the stage of severe dementia, all cognitive functions are highly impaired, such as loss of biographical memories and language inadaptability. Aggression and restlessness are also very common, which sometimes can be responses to pain, abrupt external stimuli, or circadian rhythm disturbances. In this stage, motor function is also highly compromised, and the patient is extremely dependent to perform basic tasks, such as eating, bathing, and using the bathroom.[82]

2. DENDRITIC CELLS VACCINE

2.1. DENDRITIC CELLS

Dendritic cells (DCs) are bone-marrow-derived cells that are present in blood, epithelial, interstitial, and lymphoid tissues, and are involved in adaptive immunity by presenting antigens to naïve T-helper cells. There are several types of dendritic cells, where the most relevant are the plasmacytoid DC (p-DCs), type 1 and type 2 conventional or myeloid DCs (c-DCs1 and c-DCs2), and the monocyte-derived DC (mo-DCs).[83]

2.1.1. Immunobiology

Dendritic cells emerge through a complex lineage of progenitor cells and differentiation processes. This process begins with hematopoietic stem cells (HSC) – contemporary models of lympho-myeloid haematopoiesis place the lymphoid-primed multipotent progenitors (LMPPs) at the apex of all myeloid and lymphoid lineages. After subsequent differentiation steps, LMPPs originate p-DCs, c-DCs1, c-DCs2, and monocytes. Each type of DCs results from the expression of different transcription factors of either myeloid lineages or monocytes.[83]

A. Type 1 Conventional DCs

Type 1 c-DCs result from the transcription of the IRF8, GATA2, PU.1, and BATF3 transcription factors in progenitor cells. The interaction between IRF8 and the other transcription factors regulates c-DCs1 levels, maturation, and differentiation.[83] These cells show the following immunophenotypic profile: MHC class 1, CD141(high levels), CD11b and CD11c (very low levels), CD13, and CD33. They also express TLR3, TLR9, and TLR10, which are used to recognize viral DNA and to produce type I interferons. Following antigen recognition, they activate killer T cells and type 1 helper T cells (Th1) responses through IL-12.[84,85]

B. Type2 Conventional DCs

c-DCs2 development is determined by the IRF4, GATA2, PU.1, and ZEB2 transcription factors. In contrast to c-DCs1, the development of these cells is not exclusive or majorly dependent on one transcription factor. Type 2 c-DCs express the following immunophenotypic profile: MHC class 1 and 2, CD1c, CD2, CD11b, CD11c, CD13, and CD33. These cells secrete IL-23, IL-1, IL-8, IL10, tumour necrosis factor-α (TNF-α), and IL-12 in bigger quantities than c-DCs1 when stimulated. They activate CD8+ T cells and promote Th1, Th2, and Th17 responses.[83,85]

C. Plasmacytoid DCs

Plasmacytoid DCs come by through GATA2, PU.1, IRF8, and IK2F transcription factors. The production of these DCs is dependent on the antagonistic interaction between ID2 and E2-2. ID2 downregulates E2-2, decreasing p-DCs formation. p-DCs express the following clusters of differentiation: CD45RA, CD123, CD303, CD304, and CD300A. These cells express TLR7 and TLR9, which recognize viral DNA; secrete type I and type III interferons, TNF-α, and IL-6; and prime CD4 T cells.[83]

D. Monocyte-derived DCs

These dendritic cells appear in inflammatory processes (such as eczema, psoriasis, coeliac disease, among others) and result from monocyte differentiation through MAFB and RLF4 transcription factors. Monocyte-derived DCs express CD13, CD33, CD11c, CD1c, CD1a, CD206, and CD209 on the cell's surface. The mo-DCs secrete IL-1, IL-12, IL-23, and TNF-α, and are involved in CD4 and CD8 T cells activation through antigen cross-presentation.[83]

2.1.2. Role In Alzheimer's Disease

DCs are heterogeneously distributed in several tissues and have migratory and sentinel abilities. Additionally, these cells play a crucial role in regulating both innate and adaptive immunity.[86,87]

DCs in the brain localize in meninges and the choroid plexus and show similarities with bone marrow-derived c-DCs. The presence of DCs in the brain result from the differentiation of DCs precursors that entered the CNS.[88] p-DCs and c-DCs are the most present in the brain but in an immature state. These cells only suffer maturation when environmental factors induce so. Immature DCs present great sentinel capacity, but do not

efficiently activate T cells. On the other hand, mature DCs can regulate T cell action, which can result in immunosuppressive and/or immunogenic responses.[7]

The specific role of DCs in the AD brain is not well established and there are several conflicting hypotheses. These cells' responses can be beneficial by maintaining the homeostasis of neuroimmune mechanisms; but also destructive by exposure to pathogenic Aβ, which leads to overactivation of inflammation.[86] In patients with AD, blood-derived DC precursor levels decrease and frequency of brain DCs increases in response to amyloid plaques in the neurovascular unit.[4,87] The recruitment of DCs promotes the infiltration of Aβ-sensitized T cells, which is more notorious in AD.[4,86] In a study conducted on transgenic mice, the removal of DCs enhances plaque formation, which means these cells are vital to the clearance of Aβ aggregates.[89] DCs are also able to regenerate and restore tissue function. On the other hand, Aβ exposure also causes impairments in dendritic cells' functions and immune responses, such as: decrease in brain-derived neurotrophic factor (BDNF) and MHC class II, which leads to a reduced interaction with neurons and T cells[90]; and differentiation into phenotypes with low-capacity for antigen presenting and high-expression of pro-inflammatory cytokines[91].

Therefore, DCs can be involved in pathophysiology of AD, through an overactivated immunogenic and pro-inflammatory response, which are known for enhancing neurodegenerative pathways. However, these cells can also have a protective role in AD through Aβ clearance and tissue regeneration.[4,86,90,91]

2.2. THE VACCINE

The potent antigen-presenting role of dendritic cells, the production of powerful cytokines, and their scarce numbers in tissues drew researchers to the development of DC-based vaccines for different pathologies, namely cancer, HIV/AIDS and neurodegenerative diseases.[92,93]

An immunotherapeutic alternative for AD is not a recent subject. The first attempt to target Aβ through immunization happened over two decades ago, and positive results are yet to emerge. Several scientists and experts claim that immunotherapeutic strategies should concern oligomeric and protofribrillary Aβ instead of monomers.[94]

2.2.1. Dendritic Cells Vaccines

A. Dendritic Cell Design and Production

Ex-vivo dendritic cells are the result of cultured HSC or monocytes in the presence of granulocyte-macrophage colony-stimulating factor (GM-CSF) and IL-4. The DCs are then furtherly matured by culturing them in the presence of different stimuli.[93,95]

Since these cells are generated *ex-vivo*, there are criteria and protocols to assess and control DCs quality for production of these vaccines. The release criteria and their conformity parameters are listed in **Table 3**.

B. Sensitization/Pulsing of Dendritic Cells

Dendritic cells are antigen-presenting cells and activate T cells through MHC molecules loaded with an antigen. Therefore, when generating *ex-vivo* DCs, these cells must be incubated or endogenously loaded with an antigen that is able to bind the MHC molecules and induce an immune response.[93]

C. Delivery of DCs

Immature DCs migrate less than mature DCs, which means that vaccines using this type of cells can benefit from a migration stimulant, such as inflammatory cytokines, metalloproteinases and TLR ligands.[93,96,97]

DCs are injected intradermally or directly into lymph nodes. Intradermal administration requires DCs to migrate to lymph nodes, but evidence shows that 95% of these cells do not comply with this requirement. Therefore, injecting directly into lymph and using migratory stimulants may dodge this migration barrier.[93] In the studies revised below, the DCs vaccines were administered to mice via peritoneal injection.

Table 3: Adapted from FIGDOR *et al.* Criteria used for quality control of different dendritic cells in vaccine production.[93]

<table>
<tr><td>Microbiology</td><td colspan="3">Negative for bacteria and fungus</td></tr>
<tr><td>Purity</td><td colspan="3">> 80%</td></tr>
<tr><td rowspan="2">Morphology</td><td>Immature</td><td colspan="2">Adherent
Floating
Round-shaped with elongations</td></tr>
<tr><td>Mature</td><td colspan="2">Attached
Veiled
Grouped</td></tr>
<tr><td rowspan="4">Phenotype</td><td rowspan="2">mo-DCs</td><td>Immature</td><td>MHC Class I^{+}
MHC Class II^{+}</td></tr>
<tr><td>Mature</td><td>CD83^{+}CD80^{+}CD86^{+}
MHC Class I^{+}
MHC Class II^{+}</td></tr>
<tr><td rowspan="2">c-DCs
p-DCs</td><td>Interstitial</td><td>CD14^{+} CD83^{+}CD80^{+}CD86^{+}
MHC Class I^{+}
MHC Class II^{+}</td></tr>
<tr><td>Langerhans DCs</td><td>CD1a^{+} CD83^{+}CD80^{+}CD86^{+}
MHC Class I^{+}
MHC Class II^{+}</td></tr>
<tr><td>Viability</td><td colspan="3">> 70%</td></tr>
</table>

2.2.2. Research And Development

The first vaccine for AD to enter a clinical trial used wild-type $A\beta_{42}$ peptide AN1972, QS-21 as an adjuvant, and polysorbate-80 as a stabilizer. However, despite the presence of a positive immune response and decrease in amyloid plaques and cognitive impairment, the trials had to be interrupted due to reports of meningoencephalitis (associated with a Th1 response, linked to the adjuvant). Therefore, the ideal immunotherapeutic approach to this disease, should – induce an immune response without neuroinflammation, modify disease onset, and reverse or slow down cognitive impairment. This is where DCs vaccines appear.[8,10,98,99]

A. CAO et. al (2008)

In accordance with the results of the AN1792 vaccine, CAO *et al.* developed an immunization strategy with three studies where DCs were sensitized with different Aβ peptides.

In the first study, the research team used six DCs vaccines, where the cells were pulsed with either an $A\beta_{42}$ wild-type peptide (PWT) or one of five mutant $A\beta_{42}$ peptides (PFM, PDM, PFDM, P22W, and P24M, see **Abbreviations**). The study was carried on 7 groups of BALB/c mice, where only the mutant peptides vaccines (particularly the PFDM vaccine) managed to induce an effective immune response, where generated antibodies react to human Aβ. None of the groups demonstrated presence of Th1 responses or unwanted inflammatory processes.[10]

In study 2, the vaccines contained DCs sensitized with one of four Aβ fragments: $A\beta_{25}$ PWT and $A\beta_{25}$ PFDM that represent a truncated T cell epitope; and $A\beta_{35}$ PWT and $A\beta_{35}$ PFDM that represent the complete T cell epitope. This study used 4 groups of BALB/c mice, where each group was administered with one of the 4 DCs vaccines. The mutant $A\beta_{35}$ elicits an efficient immune response, but the $A\beta_{25}$ peptides do not. This means that to be effective, DCs must be exposed to the complete T cell epitope of a mutant Aβ peptide.[10]

In study 3, the researchers vaccinated APP transgenic mice with PWT and P22W. The data obtained corroborated the previous results where mutant Aβ induces a notorious antibody response, but PWT does not.[10]

What information can we take from CAO et al.'s study? 1. Immune responses only occur if DCs are exposed to mutant Aβ peptides that represent the complete T cell epitope; 2. DC-based vaccines do not elicit Th1 immune responses and do not need an adjuvant or large quantities of antigen to create antibodies, consequently not inducing unwanted inflammatory reactions; 3. DCs can be collected from the host, thus avoiding graft-versus-host reactions (GHR); 4. Antibody levels are still detectable six months post-immunization.

B. LUO et al. (2012)

Following the positive results from the previous study, LUO *et al.* used C57BL/6 (C57) mice and PDAPP transgenic mice models for AD, where they assessed the effect of the vaccine in antibody production, Aβ loads, and cognitive function (without Th1 inflammation). This study used five vaccines: PDFM-sensitized DCs and PWT with adjuvant, which are active vaccines; and PWT-sensitized DCs, non-sensitized DCs vaccine, and PBS, which are inactive vaccines.

PDFM activates DCs, which in turn elicit immune responses, whereas PWT does not since it is a self-antigen. PWT only produces an immune response in the presence of an adjuvant. PDFM-induced antibodies showed a slower growth in C57 mice but did not

decrease. Oppositely, the adjuvant group's antibody count decreased after 21 days of vaccination, despite having showed the highest peak of antibodies. In the AD models (PDAPP mice), all groups' antibody titer decreased, but cognitive function improved significantly in both PDFM and adjuvant groups.

Cytokine profiling showed that the difference between the PDFM and adjuvant groups lied in the production of Th1-specific cytokines, such as IL-1α, IFN-γ, and TNF-α. These cytokines were absent in the PDFM group (that showed expression of G-CSF, IL-17, IL-6, and were cultured with IL-4, which enhance Th2 and inhibit Th1 responses), but were elevated in the adjuvant group. This proves that DCs vaccine do not elicit an undesirable Th1 response.

In regards of Aβ loads, both active vaccines decreased amyloid burdens in PDAPP mice. However, the DCs vaccine induces a more significant reduction in amyloid loads, despite showing lower antibody titers.

This study also assessed which mechanism of action is responsible for the positive effects of the vaccine besides the antigen-presenting and T cell-activating roles of DCs. The vaccine caused an increase in production of LXR and ABCA1, which are responsible for removing excess cholesterol (proved to be involved in AD pathogenesis).[8]

What information can we take from LUO et al.'s study? 1. DCs vaccines generate a slow, but steady, growth of antibodies; 2. DCs vaccines lower Aβ burdens and improve cognitive function, without Th1 inflammatory responses or graft-versus-host reactions; 3. DCs vaccines' mechanism of action may be linked to the LXR/ABCA1 pathway, which is responsible for removing excess cholesterol in the CNS.

C. SONG *et al.* (2020)

The previous successful results in DCs vaccine led SONG *et al.* to develop a new therapeutic vaccine for AD using an $A\beta_{42}$ peptide mutated in the 22nd amino acid (glutamate to tryptophan) – E22W42.[9] This peptide is proposed to stabilized oligomeric forms of Aβ, which are the main participants in neuronal death. By joining this peptide and the immunomodulatory component of DCs, this vaccine can be used as an immunotherapeutic vaccine for AD.[9,59] This study used 25, 35, and 42 amino acid long PWT and mutant (E22W) Aβ to sensitize DCs. The vaccines were then given to both APP/PS1 transgenic mice and C57 mice groups.[9]

E22W42 has 13 more novel T cell epitopes than the wild-type peptide, and all have strong affinities to both MHC class I and II, which are necessary to activate CD8 and CD4 T cells, respectively. As seen before, Aβ peptides only generate an immune responsible if the complete T cell epitope is present, which means they should be at least 35 amino acids long.[9,10]

The E22W42-senstized DCs vaccine (E22W42-V) produced oligomeric-specific antibodies capable of generating an efficient immune response, as well as elevated blood and brain levels of Aβ antibodies. Additionally, E22W42-sensitized DCs retain their immunomodulatory properties, unlike PWT-sensitized DCs. As seen in other DCs vaccines, E22W42-V does not elicit a Th1 response or cause inflammation. The AD mice treated with this vaccine showed memory improvement and increased LC3 (a protein involved in autophagic processes, which may regulate Aβ clearance and neurodegeneration).[9]

What information can we take from SONG et al.? 1. Aβ peptides must have a mutation and a complete T cell epitope to elicit an immune response; 2. E22W42-V generates oligomeric-specific antibodies; 3. DCs in this vaccine retain their immune functions; 4. E22W42 is a great candidate for an AD vaccine.

CONCLUSION

AD is a devastating disease that impacts the lives of millions of people worldwide, both patients and caregivers. This disease leads to severe symptoms and morbidities in patients, who develop serious cognitive impairments and other life-threatening pathologies.

In response to the lack of treatment options and the poor outcomes from drug therapies, researchers turned their eyes to immunotherapeutic alternatives to modulate the disease's course and onset more successfully and safely. Due to success cases in other pathologies, such as cancer, researchers gained interest in dendritic cells, their functions, and their promising therapeutic effects to fight AD-related symptoms.[92,100,101]

Although the amount of information concerning DCs vaccines is not very extensive, the results from three studies showed promising results in vaccines administered to animal models. This vaccine consists of DCs pulsed with mutant Aβ peptides, which then activate the immune system and generate specific antibodies able to neutralize the neurotoxic events evoked by Aβ. The positive results documented with this type of vaccines showed that there is a promising path in AD therapy. These results may lead to a much-needed therapeutic alternative that is cheaper, more efficient, and more easily administered.[8–10] The improvements in cognitive function and Aβ clearance demonstrated in animal models of AD with the E22W42-V, along with the proven safety of this type of vaccines may be the starting step to achieve a therapeutic answer that puts a stop in the crushing consequences of this disease.

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