UNIVERSIDADE D
COIMBRA

Maria João Borges de Almeida e Costa

**Relatórios de Estágio e Monografia intitulada "The Potential of Chronotherapy in Rheumatoid Arthritis" referentes à Unidade Curricular "Estágio", sob a orientação, do Dr. Paulo Viegas de Carvalho, da Dra. Dina Cordeiro Lopes e da Professora Doutora Alexandrina Ferreira Mendes 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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Coimbra, 02 de setembro de 2019.

Maria João Costa

(Maria João Costa)

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# Parte I: Relatório de Estágio em Farmácia Comunitária

## Lista de Abreviaturas

**FFUC** - Faculdade de Farmácia da Universidade de Coimbra

**FSC** - Farmácia Santa Clara

**MICF** - Mestrado Integrado em Ciências Farmacêuticas

**MNSRM** - Medicamento Não Sujeito a Receita Médica

**PIM** - Preparação Individualizada da Medicação

**SWOT** - *Strengths, Weaknesses, Opportunities, Threats*

# I. Introdução

O farmacêutico é um agente de saúde pública a quem compete executar todas as tarefas relacionadas com o medicamento[1]. No exercício da sua atividade em farmácia comunitária deve-se realçar o facto de ser este o principal elo de ligação com a população em geral, constituindo muitas vezes o primeiro contacto do utente antes da procura por aconselhamento médico. É fulcral que, no desempenhar das suas funções, o farmacêutico colabore estritamente com todos os profissionais de saúde promovendo uma utilização segura, eficaz e racional dos medicamentos de uso humano e veterinário, assim como de dispositivos médicos[1]. Como resultado da extensa formação académica, é este especialista do medicamento quem detém os conhecimentos científicos adequados ao aconselhamento e indicação de medicamentos não sujeitos a receita médica (MNSRM) e outros produtos de saúde, e é responsável por assegurar que o doente recebe toda a informação correta, com qualidade máxima e em harmonia com as boas práticas de farmácia[1]. É também um dever do farmacêutico dispensar os medicamentos em cumprimento da prescrição médica, com exceção das situações em que os seus conhecimentos permitam satisfazer de uma melhor forma as relações benefício/risco e benefício/custo para o utente[1].

Sendo a farmácia comunitária uma especialidade farmacêutica que requer a integração e interligação dos conhecimentos adquiridos nas mais variadas unidades curriculares do plano de estudos do Mestrado Integrado em Ciências Farmacêuticas (MICF) da Faculdade de Farmácia da Universidade de Coimbra (FFUC), faz todo o sentido a realização deste estágio curricular para culminar cinco anos de intensa formação teórica e prática. Para além de permitir por em prática os conhecimentos adquiridos, este estágio permite um contacto privilegiado com o mundo do trabalho, contribuindo não só para um crescimento profissional enquanto futuros farmacêuticos, mas também para um crescimento pessoal.

Escolhi a Farmácia Santa Clara (FSC) como local de estágio para aprimorar os meus conhecimentos devido à familiaridade que já tinha adquirido com a equipa da direção técnica num estágio extracurricular realizado anteriormente. O presente relatório de estágio apresenta-se assim sob a forma de uma análise SWOT (*Strengths, Weaknesses, Opportunities, Threats*) com o objetivo de identificar, internamente, os pontos fortes e os pontos fracos do estágio na FSC e, externamente, as oportunidades e as ameaças sentidas no decorrer desta formação. Este período de aprendizagem teve início a 1 de abril de 2019 e término a 12 de julho do mesmo ano, perfazendo um total de 670 horas de trabalho sob a orientação do Dr. Paulo Viegas de Carvalho.

# II. Análise SWOT

## A. Pontos Fortes (*Strenghts*)

Localização e Horário de Funcionamento

A FSC encontra-se na Rua Central da Mesura dentro do edifício do Intermarché constituindo um ponto forte a proximidade com este estabelecimento comercial e com diversas unidades de saúde tais como os Centros de Saúde de Santa Clara e São Martinho do Bispo e o Hospital dos Covões, parte integrante do Centro Hospitalar e Universitário de Coimbra. A localização fora dos grandes centros urbanos da cidade de Coimbra permite que esta farmácia não funcione apenas como local de passagem para os utentes, havendo uma grande taxa de utentes habituais e fidelizados provenientes quer da zona, quer de aldeias vizinhas.

O horário de atendimento da FSC decorre de segunda-feira a sábado entre as 9h e as 21h, e aos domingos e feriados entre as 10h e as 20h. Para além das vantagens claras que este horário alargado traz para os utentes, do ponto de vista de estagiária este é um ponto forte fulcral por me ter permitido estagiar em diferentes períodos do dia. A alternância entre abertura e fecho de farmácia e o estágio aos fins de semana e feriados permitiu contactar com diferentes realidades de fluxo de utentes e diferentes quadros clínicos, colocando à prova a minha capacidade de adaptação e resposta rápida às diversas situações.

Equipa Técnica

Considero que o ambiente e as relações estabelecidas entre o estagiário e a equipa que o acolhe são essenciais para a sua aprendizagem durante o período de estágio. Toda a equipa técnica da FSC constitui assim um ponto forte do meu estágio pela simpatia, entreajuda e profissionalismo demonstrados ao longo deste período. Destaco o auxílio prestado durante os primeiros atendimentos e a disponibilidade para intervirem espontaneamente sempre que as situações assim o exigissem, tal como a motivação e incentivo para colocar o estagiário no atendimento ao público dando-lhe todos os conhecimentos e independência necessários. Deste modo, a equipa da FSC, contribuiu não só para o meu enriquecimento enquanto farmacêutica, mas também enquanto pessoa pelas relações de amizade estabelecidas.

Plano de Estágio

Desde o primeiro dia de estágio que o estagiário é posto em contacto com as diversas tarefas desempenhadas pelo farmacêutico na farmácia comunitária. A não existência de um plano de estágio estrito constitui um ponto forte desta minha formação, dado que permitiu a alternância entre diversas funções e a aquisição simultânea de inúmeros conhecimentos de um modo interligado.

Tendo iniciado o estágio a 1 de abril, umas das primeiras situações com que contactei foi a organização mensal do receituário para efeitos de faturação. Antes deste ser enviado ao Centro de Conferência de Faturas até ao dia 5 de cada mês, todo o receituário, exceto as receitas eletrónicas, é conferido e organizado por lotes. Esta aprendizagem sensibilizou-me desde logo para a importância de validar corretamente todas as prescrições médicas manuais, atendendo aos medicamentos prescritos, dados do utente, dados do médico prescritor e validade da receita. Desde logo, fui também familiarizada com o *software* Sifarma 2000® no que diz respeito à criação e receção dos diversos tipos de encomendas. Nesta tarefa, foi-me incutida a importância da verificação dos prazos de validade e da diferença entre o preço de venda ao público e o preço de venda à farmácia, o que tem um grande impacto nas margens de lucro obtidas. No decorrer do estágio contactei também com controlos periódicos de *stock* onde se procede à contagem física dos produtos e avaliação da sua conformidade com o número de unidades e o prazo de validade registados informaticamente. Uma tarefa pouco comum na FSC, mas a qual tive a oportunidade de desempenhar, é a preparação de medicamentos manipulados. No desempenhar desta tarefa procedi à preparação de uma pomada composta por 60 g de clobetasol, 12 g de ácido salicílico e 30 g de vaselina para aplicação tópica em lesões de psoríase. Podendo assim colocar em prática conhecimentos adquiridos em Farmácia Galénica.

O atendimento ao público foi, sem dúvida, a tarefa mais enriquecedora e satisfatória do meu estágio. Desde o primeiro dia de estágio que a equipa da FSC incentiva o estagiário a assistir aos atendimentos de forma a que se familiarize com o Sifarma 2000® bem como com os utentes e as diversas situações que os levam à farmácia. Ainda durante a primeira semana iniciei atendimentos ao público, acompanhada de um membro da equipa para me auxiliar sempre que necessário. E ao fim de pouco tempo já tinha depositado em mim conhecimentos suficientes e confiança para iniciar de forma independente estes mesmos atendimentos. Considero esta rapidez de evolução e a independência dada ao estagiário um ponto forte para acelerar e melhorar a sua aprendizagem, dado que é mais fácil aprender a fazer do que apenas a observar. Com o decorrer do tempo senti que houve um aperfeiçoar dos conhecimentos

adquiridos durante o plano de estudos do MICF e que consegui por em prática os princípios e valores que me foram incutidos. Para além disso, desenvolvi competências relacionadas com as relações interpessoais, espírito crítico e autonomia que considero fundamentais enquanto futura farmacêutica.

Serviços

A FSC visa o bem-estar e a manutenção da saúde dos seus utentes, e como tal dispõe de uma vasta gama de serviços como a determinação de parâmetros bioquímicos e fisiológicos (colesterol total, triglicéridos, glicémia e pressão arterial), a administração de injetáveis e consultas de nutrição e podologia. A participação em alguns destes serviços, nomeadamente na determinação de colesterol total, glicémia e pressão arterial, foi um ponto forte do meu estágio por poder aplicar diretamente os meus conhecimentos acerca da realização destas medições, assim como poder esclarecer eventuais dúvidas acerca da medicação e/ou patologia e aconselhar o utente de acordo com os resultados obtidos.

*Robot* e Caixa Automática

Uma grande vantagem da FSC é existência de recursos tecnológicos que permitem aumentar e melhorar a sua eficiência e eficácia – o *robot* BD Rowa Smart® e a caixa Cashlogy *by* Azkoyer. O *robot* está diretamente ligado ao Sifarma 2000® sendo um ponto forte pela sua capacidade de facilitar quer o armazenamento, quer a dispensa de medicamentos. Para além de agilizar estes processos que poderiam ser muito morosos, também rentabiliza o espaço físico da farmácia e liberta colaboradores para outras tarefas. Outra grande vantagem deste sistema de armazenamento é a minimização de potenciais erros, tal como troca de medicamentos e/ou dosagens, que poderiam ocorrer durante a dispensa dos medicamentos ao utente. Relativamente à caixa automática, esta constitui um ponto forte por agilizar o atendimento ao público no que diz respeito à devolução do troco ao utente eliminando potenciais erros, para além disso regista todos os movimentos de cada operador e facilita a gestão dos totais de caixa diários.

## B. Pontos Fracos (*Weaknesses*)

Lacunas no Aconselhamento Farmacêutico

É um facto que nem todos os conteúdos com os quais o farmacêutico se depara no dia a dia possam ser incluídos no plano de estudos do MICF e que o próprio estágio constitui uma

etapa fundamental do processo de formação do aluno, no entanto senti que existem algumas lacunas no plano de estudos que, consequentemente, se fazem sentir quando nos deparamos com o mundo real do trabalho. No meu caso, a mais notória foi a falta de conhecimentos que permitissem um eficaz aconselhamento na área dos medicamentos e produtos veterinários. Apesar da existência de uma unidade curricular subordinada a este tema, na minha opinião, a abordagem que nela é feita não vai nada de encontro à realidade da prática farmacêutica e aos conhecimentos que nos são exigidos numa farmácia comunitária. Isto leva a que o estagiário não tenha confiança na indicação destes produtos e que tenha que frequentemente procurar ajuda junto dos colaboradores da farmácia quando questionado acerca destes.

## C. Oportunidades (*Opportunities)*

Acompanhamento Farmacoterapêutico

Com a realização deste estágio tornou-se evidente a grande oportunidade que há de instituir o acompanhamento farmacoterapêutico de utentes nos serviços disponibilizados pela farmácia. Este serviço consiste em associar a medicação do utente a cada um dos seus problemas de saúde de modo a avaliar a efetividade e segurança da terapêutica, assim como detetar eventuais problemas de saúde não tratados, realizando um plano das intervenções adequadas a cada situação. Na FSC, o elevado número de utentes fidelizados e polimedicados para diversas patologias constitui uma das principais razões para o benefício da instituição deste serviço. Para além de vantajoso para a farmácia e para os utentes, este serviço seria também uma mais-valia no plano de estágio por permitir que o estagiário demonstrasse e integrasse os conhecimentos desenvolvidos no MICF de uma forma que o atendimento ao balcão muitas vezes não permite.

Preparação Individualizada da Medicação

A preparação individualizada da medicação (PIM) é um dos mais recentes serviços que podem ser prestados nas farmácias comunitárias. A PIM consiste na organização da medicação (formas farmacêuticas sólidas) do utente, de acordo com a posologia descrita, em caixas dispensadoras próprias[2]. Este serviço constitui assim um auxílio ao utente na correta administração dos medicamentos e uma das soluções disponíveis para minimizar a não adesão não intencional à terapêutica, principalmente em doentes com regimes terapêuticos complexos e doentes com dificuldade no uso de medicamentos[2]. A existência de uma população envelhecida, polimedicada e com elevada incidência de doenças crónicas faz deste

serviço uma oportunidade de intervenção junto desta, melhorando não só a qualidade de vida dos utentes assim como a intervenção social e a qualidade dos serviços disponibilizados pela farmácia.

## D. Ameaças (*Threats*)

Medicamentos Esgotados

Desde o início do estágio que fui confrontada com o problema dos medicamentos esgotados. Esta situação externa à farmácia constitui uma clara ameaça ao seu pleno funcionamento, afetando não só as potenciais vendas que não se realizaram, mas também a relação com os utentes. Foram frequentes, durante todo o período de estágio, as ocasiões em que não pude satisfazer as necessidades dos utentes, algumas destas gerando conflitos pelo facto do utente não entender que a situação em nada dependia dos colaboradores da farmácia. Este problema exigia assim uma atenção constante na gestão e criação de encomendas e, quando possível, a racionalização do reduzido número de medicamentos que nos eram fornecidos. A título de exemplo, destaco o facto de aquando do início do estágio a Aspirina® GR 100 mg já se encontrar esgotada há algum tempo, problema que só viria a ficar resolvido em meados de junho.

Locais de Venda de Medicamentos Não Sujeitos a Receita Médica

Uma evidente ameaça para todas as farmácias comunitárias é existência dos locais de venda de MNSRM. Durante o meu estágio na FSC também foi notável esta ameaça através dos atendimentos que não se concretizaram por o utente referir que em determinado local tinha acesso ao mesmo produto a preços mais acessíveis. A aquisição de produtos a um preço inferior, devido ao seu maior poder de compra, constitui assim a grande vantagem que estes locais proporcionam aos utentes, impactando na viabilidade económica das farmácias. No entanto, a falta de profissionais qualificados e com formação adequada para poder proceder ao aconselhamento com máxima qualidade e segurança também deveria constituir um ponto fulcral na decisão do utente. Deste modo, é imperioso diferenciar o atendimento feito na farmácia, proporcionando ao utente todas as informações necessárias para assegurar uma correta e segura utilização dos medicamentos e produtos de saúde, destacando o importante e insubstituível papel do farmacêutico na comunidade.

# III. Casos Clínicos

A cada atendimento há sempre a oportunidade de demonstrar e por em prática os conhecimentos teóricos previamente adquiridos, havendo uma maior potencialidade no caso da indicação farmacêutica do que na dispensa de medicamentos sujeitos a receita médica. A título de exemplo, seguem três casos com os quais me deparei durante o período de estágio e que me permitiram dar provas dos conhecimentos adquiridos durante estes anos de formação.

## Caso I

Utente do sexo feminino, com cerca de 40 anos, apresenta-se na farmácia com a mucosa bucal e língua inchadas e cobertas de pequenas lesões avermelhadas que lhe conferem dor e a impossibilitam de comer. Refere que já se encontra neste estado há cerca de uma semana e que, por indicação de um médico conhecido, está a fazer Myconstatin® 100.000 U.I. / ml Suspensão oral, indicado no tratamento de candidíase oral, mas a situação tem piorado. Após descartar fatores de risco para candidíase orofaríngea, tal como corticosteroides inalados, e avaliar as lesões, expliquei à utente que não havia indicação para continuar o tratamento e que o mais aconselhável seria utilizar apenas um produto indicado para aftas e úlceras bucais. Recomendei a utilização da gama Bexident® AFTAS que, devido à sua tecnologia *Advanced Hyalurofilm Tech*, cria uma película por cima da lesão que alivia a dor, protege contra agentes externos, hidrata os tecidos e facilita a cicatrização[3,4].Dentro dos produtos disponíveis, a utente optou por levar o colutório para bochechar e assim permitir o contacto do produto com toda a cavidade bucal e o gel para aplicar diretamente nas lesões. Numa posterior visita à farmácia, a utente demonstrou a sua satisfação com o aconselhamento dado e a fidelização com a marca, renovando o *stock* dos produtos para a eventualidade de necessitar novamente.

## Caso II

Um utente dirigiu-se à farmácia a solicitar Imodium Rapid® para a sua esposa que se encontrava com diarreia. Este é um MNSRM contendo 2 mg de loperamida, um fármaco antidiarreico que vai modificar a motilidade intestinal e favorecer a reabsorção de água no intestino. A familiaridade demonstrada pelo utente para com este medicamento fazia com que, à primeira vista, este lhe parecesse uma boa opção terapêutica. No entanto, quando questionado acerca da duração do quadro clínico e sobre a presença de outros sinais e

sintomas, como dores abdominais, febre ou presença de sangue nas fezes, referiu que a situação já se prolongava há quatro dias e que era acompanhada de um forte desconforto abdominal. Tendo em conta esta nova informação, desaconselhei a utilização do antidiarreico que poderia mascarar uma patologia subjacente e recomendei que se dirigisse com a esposa ao médico, dando ênfase às medidas não farmacológicas que deveria iniciar de imediato, tais como a hidratação e reposição de eletrólitos.

## Caso III

Utente do sexo feminino, cerca de 70 anos, foi à farmácia levantar a sua medicação habitual fazendo-se acompanhar de uma prescrição médica. Em conversa durante o atendimento refere que o medicamento que toma para o colesterol lhe causa intensas dores musculares, sobretudo nas pernas, e que o médico já tem conhecimento e até já o substituiu por outro, mas as dores continuam. Segundo a prescrição, a terapêutica para o colesterol elevado desta senhora era feita com uma estatina, uma classe de fármacos que inibe a síntese endógena de colesterol através da sua ação na enzima hidroximetilglutaril-coA redutase e que tem como efeito secundário a toxicidade muscular. Aconselhei que o ideal seria falar novamente com o médico para que em conjunto pudessem retirar as estatinas da terapêutica e procurar outra opção eficaz e com um melhor perfil de segurança. Nesse sentido, apresentei à utente o suplemento alimentar Cholesfytol®. Este é constituído por monacolina K de 2ª geração extraída da levedura de arroz vermelho, que vai reduzir a biossíntese hepática de colesterol ao mesmo tempo que apresenta menor risco de interações e efeitos secundários, entre eles dores musculares[5]. Para além desta ação direta na diminuição dos níveis de colesterol, tem um efeito antioxidante devido à presença de hidroxitirosol, protegendo o colesterol LDL de danos oxidativos e consequentemente atrasando a progressão da aterosclerose [5]. Informei também que este efeito benéfico é conseguido com a toma de 1 comprimido por dia ao deitar, de modo semelhante às estatinas[5].

# IV. Conclusão

O estágio curricular em farmácia comunitária assume-se como o culminar de cinco anos de intensa formação teórica e prática, que permite por em prática, de modo interligado, todos os conhecimentos técnicos e científicos adquiridos ao longo deste percurso académico, assim como melhorá-los. Cada vez mais a farmácia constitui o primeiro local de contacto do utente em caso de necessidade, devido à disponibilidade e facilidade em obter as informações desejáveis fornecidas por profissionais altamente qualificados. Assim, através da exposição do estudante à realidade do mundo de trabalho, este estágio contribui para a sua consciencialização acerca do papel do farmacêutico na comunidade, as oportunidades e dificuldades com que se depara no dia a dia e quais as mudanças necessárias para continuar a dignificar o bom nome desta profissão.

Tudo isto se aplica ao meu estágio na FSC, onde desenvolvi competências não só científicas, mas também de caráter pessoal que considero essenciais ao desempenho de uma atividade farmacêutica consciente e com foco na saúde e bem-estar do utente. É com enorme satisfação que revejo o meu percurso de estagiária e agradeço à equipa técnica da FSC por proporcionarem esta oportunidade aos estudantes e os prepararem para um futuro profissional de excelência.

# V. Bibliografia


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# Parte II: Relatório de Estágio em Assuntos Regulamentares do Medicamento

## Lista de Abreviaturas

**AIM** - Autorização de Introdução no Mercado

**CAM** - Comissão de Avaliação de Medicamentos

**CTS** - *Communication and Tracking System*

**DAM** - Direção de Avaliação de Medicamentos

**DRHFP** - Direção de Recursos Humanos, Financeiros e Patrimoniais

**FFUC** - Faculdade de Farmácia da Universidade de Coimbra

**FI** - Folheto Informativo

**GestProc** - Base de Dados de Gestão de Processos

**GIMED** - Base de Dados de Gestão de Informação de Medicamentos

**GPRen** - Base de Dados de Gestão de Processos de Renovação

**INFARMED, I.P.** - Autoridade Nacional do Medicamento e Produtos de Saúde I.P.

**MICF** - Mestrado Integrado em Ciências Farmacêuticas

**MUH** - Medicamentos de Uso Humano

**RCM** - Resumo das Caraterísticas do Medicamento

**SMUH-ALTER** - Plataforma de Submissão de Pedidos de Alteração do Sistema de Gestão de Medicamentos de Uso Humano

**SWOT** - *Strengths, Weaknesses, Opportunities, Threats*

**UAC** - Unidade de Avaliação Científica

**UEC** - Unidade de Ensaios Clínicos

**UIM** - Unidade de Introdução no Mercado

**UMM** - Unidade de Manutenção no Mercado

# I. Introdução

Os Assuntos Regulamentares constituem uma potencial área profissional para o desenvolvimento da atividade farmacêutica. Dotados de extensos conhecimentos em inúmeras vertentes do medicamento, os farmacêuticos exercem a sua atividade fundamental nos processos de desenvolvimento, registo, acesso ao mercado e monitorização da utilização de medicamentos e dispositivos médicos, assim como na informação e apoio aos profissionais de saúde[1].

A Faculdade de Farmácia da Universidade de Coimbra (FFUC) possibilita aos alunos do Mestrado Integrado em Ciências Farmacêuticas (MICF) a oportunidade única de estagiar na Autoridade Nacional do Medicamento e Produtos de Saúde, I.P. (INFARMED, I.P.). Posto isto, decidi optar pela Direção de Avaliação do Medicamento no INFARMED, I.P., não só pela preponderância da área dos assuntos regulamentares do medicamento no mercado farmacêutico, como também pela oportunidade de adquirir uma visão do circuito do medicamento enquanto parte integrante de uma agência regulamentar.

O presente relatório apresenta-se sob a forma de uma análise SWOT (*Strengths, Weaknesses, Opportunities, Threats*) com o objetivo de retratar o estágio curricular em Assuntos Regulamentares do Medicamento, identificando, internamente, os seus pontos fortes e pontos fracos e, externamente, as oportunidades e as ameaças sentidas. Este estágio decorreu no INFARMED I.P., de 7 de janeiro de 2019 a 29 de março do mesmo ano, sob orientação da Dr.ª Dina Cordeiro Lopes.

# II. INFARMED, I.P.

O INFARMED, I.P. é o instituto público português responsável pela regulação, supervisão e fiscalização, segundo os mais elevados padrões de proteção da saúde pública, da qualidade, eficácia e segurança dos setores dos medicamentos, dispositivos médicos e produtos cosméticos[1]. Sob tutela do Ministério da Saúde e com jurisdição sobre todo o território nacional, o INFARMED, I.P. encontra-se sediado no Parque da Saúde de Lisboa onde, com um elevado nível de organização, se estrutura em cinco órgãos e treze unidades orgânicas, estando estas subdivididas consoante desempenhem funções de negócio ou de suporte (Figura 1)[2,3].

A Direção de Avaliação de Medicamentos (DAM) constitui uma das unidades orgânicas com funções de negócio e é integrada por quatro grandes unidades: a Unidade de Ensaios Clínicos (UEC), a Unidade de Introdução no Mercado (UIM), a Unidade de Manutenção no Mercado (UMM) e a Unidade de Avaliação Científica (UAC)[3]. Durante o meu estágio curricular fui integrada na UMM, que desempenha as suas funções na fase posterior à concessão de uma Autorização de Introdução no Mercado (AIM), responsabilizando-se pela gestão de processos de Alterações, Renovações e Revogações de Medicamentos de Uso Humano (MUH), de forma a assegurar a sua manutenção no mercado farmacêutico[4]. Dentro da UMM os colaboradores são organizados em diferentes equipas de trabalho, tendo o meu estágio decorrido na equipa dedicada à gestão das alterações aos termos de AIM submetidas para medicamentos aprovados por procedimento nacional.

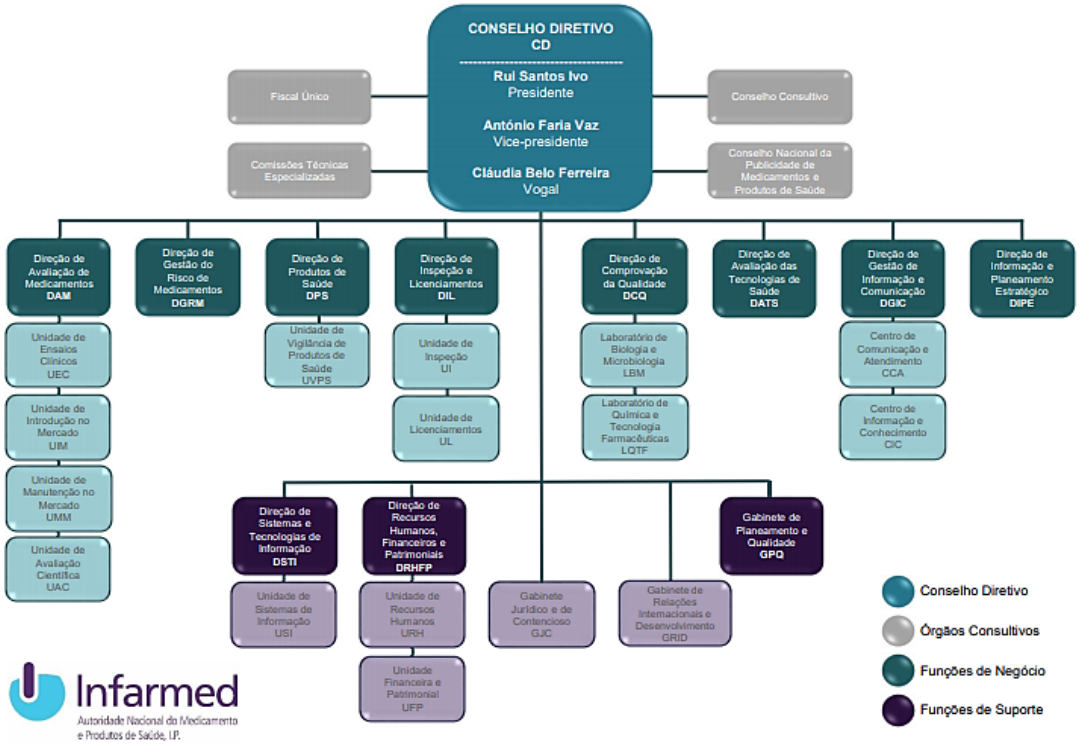


**Figura 1** – Organograma do INFARMED, I.P.[3]

# III. Análise SWOT

## A. Pontos Fortes (*Strenghts*)

Acolhimento Inicial e Integração na Equipa de Trabalho

No primeiro dia de estágio curricular no INFARMED, I.P. fomos desde logo acolhidos pelo Dr. José Viana da Direção de Recursos Humanos, Financeiros e Patrimoniais (DRHFP). Neste primeiro contacto, foram-nos dadas a conhecer as instalações físicas e foi providenciada

uma curta formação acerca da organização estrutural e funcional da instituição, assim como das normas e políticas em vigor. Posteriormente, cada estagiário foi encaminhado para o seu orientador de estágio e integrado no respetivo departamento. No meu caso em particular, fui encaminhada para a DAM-UMM-Procedimentos Nacionais onde me foram atribuídos um *email* institucional, um número mecanográfico e um computador de trabalho através do qual teria acesso à rede e às plataformas informáticas imprescindíveis à execução das minhas funções.

Durante esta primeira semana, assisti a um conjunto de formações teóricas que me permitiram recordar conceitos relacionados com os tipos de procedimentos de registo dos MUH e os possíveis tipos de alterações aos termos de AIM, que se mostraram essenciais ao desempenho pleno das funções que me foram atribuídas. Paralelamente, fui familiarizada com as diversas plataformas informáticas utilizadas como ferramentas de trabalho na DAM tais como o SMUH-ALTER (plataforma de submissão de pedidos de alteração do sistema de gestão de MUH), o GIMED (base de dados de Gestão de Informação de Medicamentos), o CTS (*Communication and Tracking System*), o GestProc (base de dados de Gestão de Processos), o GPRen (base de dados de Gestão de Processos de Renovação), entre outras. Considero assim a primeira semana de estágio um ponto forte, na medida que foi bastante esclarecedora acerca das funções e do objetivo da nossa presença, e me permitiu adquirir conhecimentos fundamentais ao pleno exercício das tarefas que me foram posteriormente concedidas.

Funções Desempenhadas

O meu estágio na DAM-UMM decorreu sob a função de gestor de processos regulamentares, a qual me permitiu estudar e analisar diferentes processos de alteração aos termos de AIM, alargando os meus conhecimentos nesta área. Como tal, qualquer tarefa por mim desempenhada no decorrer do estágio constitui um ponto forte da minha formação.

A gestão de cada processo de alteração inicia-se pela sua validação, onde se procede à análise do pedido submetido através da verificação dos dados do requerente, o comprovativo do pagamento da taxa de submissão do pedido, o tipo de alteração, a informação constante no GIMED acerca do medicamento e a documentação de suporte submetida (formulário do pedido, requerimento, declaração de autorização de uso de *email*, cópia das páginas relevantes da *guideline,* entre outros). Esta fase é da exclusiva responsabilidade do gestor do processo, ao qual cabe a função de pedir elementos de validação sempre que necessário e de considerar, em última instância, o pedido como validado ou não validado. Encontrando-se tudo isto em conformidade, cabe aos avaliadores externos ou internos iniciar a fase seguinte de avaliação.

Como o próprio nome indica, nesta fase vai-se avaliar o pedido da alteração e a sua conformidade com os respetivos módulos do dossiê de AIM, assim como o impacto da alteração na qualidade, eficácia e segurança do medicamento. Consoante o parecer do avaliador, positivo ou negativo, a alteração pode ser aceite ou não, respetivamente.

Outras funções que também são responsabilidade do gestor dos processos e as quais tive oportunidade de desempenhar incluem estabelecer contacto com o requerente responsável pelo pedido de alteração, quer seja para esclarecer questões, quer para atualizar acerca do estado do pedido; atualizar e/ou corrigir os dados constantes nas bases de dados referentes ao medicamento, assim como rever e corrigir textos para integrar no Resumo das Caraterísticas do Medicamento (RCM) e/ou no Folheto Informativo (FI) do medicamento.

Competências Desenvolvidas

Foram várias as alterações submetidas que me permitiram aplicar conhecimentos teóricos previamente adquiridos no plano curricular do MICF, assim como desenvolver novas competências fundamentais para vingar no mercado de trabalho. Considero entre as mais relevantes as competências informáticas, dado que a gestão dos processos era feita exclusivamente através da consulta, atualização e operacionalização de bases de dados internas, plataformas de gestão nacionais e europeias, e plataformas para a submissão e partilha de documentos. É de realçar também, o aprofundamento do meu conhecimento relativo à tipificação e categorização de alterações aos termos de AIM, assim como às legislações e políticas do medicamento vigentes. Neste contexto, é ainda de destacar a aptidão adquirida na extração rápida de informação a partir de documentos técnicos e científicos, muitas vezes extensos e confusos aos olhos de um estagiário inexperiente.

Responsabilidade

Um ponto forte que contribuiu diretamente para o meu crescimento enquanto profissional autónomo e capaz foi o elevado grau de responsabilidade das funções desempenhadas. No início, foi atribuído a cada estagiário um conjunto de processos pelos quais este era inteiramente responsável por finalizar. No decorrer da validação dos meus processos todas as decisões eram fundamentalmente tomadas por mim, estando os colaboradores à disposição caso houvesse necessidade, assim como o estabelecimento de contactos com os requerentes e titulares de AIM. Todo este grau de autonomia que a equipa

nos proporcionava incutiu um elevado sentido de responsabilidade que, a meu ver, é uma valência indispensável no mundo do trabalho.

Reunião da Comissão de Avaliação de Medicamentos

Um ponto forte do estágio nesta entidade regulamentar foi a oportunidade dada aos estagiários da DAM de assistirem a uma reunião da Comissão de Avaliação de Medicamentos (CAM). Periodicamente e segundo um calendário previamente definido, estas reuniões destinam-se à análise de pareceres de pedidos de AIM, alterações ou renovações, elaborados por peritos de diversas áreas onde se destaca a presença de Farmacêuticos. Nos três dias úteis que precedem a reunião é enviada a ordem de trabalhos a todos os que nela participarem, para que analisem cada situação e possam manifestar a sua concordância ou não com a decisão. Durante a reunião são colocadas questões e esclarecidas dúvidas quando surgem pareceres negativos ou discordantes que impeçam a aprovação do processo de um dado medicamento. Foi de destacar a presença do Professor Dr. Francisco Veiga e do Professor Dr. João José de Sousa como peritos farmacêuticos, contribuindo para uma maior sensibilização acerca do papel do farmacêutico nesta área e da grande responsabilidade que as suas funções acarretam.

## B. Pontos Fracos (*Weaknesses*)

Fluxo de Trabalho Irregular

O elevado número de processos de alteração de procedimentos nacionais pendentes de validação aliado aos constantes atrasos por parte dos titulares de AIM na submissão de respostas aos pedidos de elementos realizados resultou na recorrente acumulação de processos não resolvidos nos quais não podia intervir. Estes períodos eram alternados com fases de intenso trabalho em que, por diversos motivos, tais como a resposta simultânea de vários titulares, a execução das tarefas e a sua conclusão se prolongava por uma série de dias. Esta constante irregularidade no fluxo de trabalho foi um ponto fraco notável que, apesar dos esforços da equipa, não conseguiu ser contornado.

Falha nos Meios Informáticos

No início do estágio o atraso que houve em conceder acessos aos estagiários às diversas plataformas internas condicionou em muito o meu trabalho, sendo impossibilitada de durante esse período realizar as funções que me tinham sido atribuídas. Uma vez concedidos todos os acessos, foi o mau funcionamento dos meios eletrónicos, sobretudo dos

computadores, uma das causas que contribuía para o atraso no seguimento dos processos. Foram inúmeras as vezes em que estes, ao bloquearem sem razão aparente, comprometiam o trabalho que estava na altura a ser realizado. Adicionalmente, também os programas internos apresentavam algumas limitações técnicas, destacando-se a lenta capacidade de processamento do GIMED e a impossibilidade de carregar ficheiros no SMUH-ALTER com tamanho superior a 10 Mb. Estas falhas constituem um ponto fraco desta minha formação, devendo por isso ser devidamente revistas e atualizadas de forma a garantir o máximo de celeridade no tratamento dos processos submetidos.

## C. Oportunidades (*Opportunities*)

Medidas de Minimização de Ruturas de *Stock* de Medicamentos

É de conhecimento público o problema dos medicamentos esgotados que o país atravessa. Após o estágio no INFARMED, I.P., identifiquei como uma oportunidade de intervenção a implementação de medidas para prevenir ruturas de abastecimento do mercado e assim assegurar o acesso regular da população aos medicamentos. Ao estagiar na UMM deparei-me com uma série de alterações pendentes de validação e/ou avaliação, algumas delas já submetidas há alguns anos. Este atraso na gestão de processos de alterações de AIM pode colocar em risco o pleno funcionamento das indústrias farmacêuticas, podendo mesmo levar a uma quebra de produção dos medicamentos e consequente rutura de *stock*. Neste sentido, e dado que muitos destes atrasos se devem ao elevado número de pedidos de alteração e reduzido número de meios humanos para os gerir, o INFARMED, I.P. tem a oportunidade de contornar esta situação através da implementação de medidas que tornem a gestão destes processos mais célere. Uma medida facilmente identificável no decorrer do meu estágio é a introdução do conceito de gerir as alterações segundo a cronologia da sua submissão.

## D. Ameaças (*Threats*)

Falta de Recursos Humanos

Não estando sob influência direta da entidade de acolhimento, a falta de recursos humanos em número suficiente para apoiar e orientar os estagiários constitui uma ameaça ao pleno funcionamento da instituição. Sendo o seu número limitado e a sobrecarga de trabalho notória, os recursos humanos muitas vezes se demonstraram como insuficientes para auxiliar os estagiários da melhor maneira, dispensando-lhes a atenção necessária ao pleno desempenho

das suas funções. Esta falha foi ainda mais notória dado o elevado número de estagiários que era necessário orientar enquanto, simultaneamente, tentavam concluir os seus processos na calendarização prevista. Não obstante, reconheço que nenhum esforço faltou aos colaboradores da equipa para que com a maior disponibilidade nos fossem dadas as explicações necessárias e esclarecidas dúvidas, não tendo, porém, sido suficiente.

# IV. Conclusão

O estágio curricular no INFARMED, I.P. foi uma das experiências mais enriquecedoras e prestigiantes do meu percurso académico. A passagem pela Unidade de Manutenção no Mercado da Direção de Avaliação de Medicamentos foi uma enorme oportunidade de aprendizagem, na qual desenvolvi novos conhecimentos e aprimorei os já existentes na área dos assuntos regulamentares do medicamento. Considero o grau de autonomia concedido e o elevado sentido de responsabilidade nas funções desempenhadas como sendo dois dos pontos fulcrais que me preparam para o desempenho das minhas funções como futura farmacêutica.

É de louvar a oportunidade única que a FFUC fornece aos seus alunos de poderem contactar com esta possível saída profissional no decorrer do seu estágio curricular, assim como a constituição do plano de estudos do MICF que, comtemplando a unidade curricular de Assuntos Regulamentares do Medicamento, os prepara para uma mais célere e eficaz integração nas equipas de trabalho do INFARMED, I.P..

É assim com enorme satisfação que revejo o meu percurso de estagiária e agradeço à equipa da DAM-UMM por, durante três meses, me acompanharem e proporcionarem conhecimentos e valores que me preparam com excelência para o mercado profissional que me espera.

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# Parte III: Monografia

# "The Potential of Chronotherapy in Rheumatoid Arthritis"

## List of Abbreviations

**6M** - 6-Methyl

**ACPA** - Anti-Citrullinated Protein Antibody

**ACR** - American College of Rheumatology

**ACR20** - At Least 20 % Improvement in RA Symptomatology According to ACR Criteria

**ACR50** - At Least 50 % Improvement in RA Symptomatology According to ACR Criteria

**ACR70** - At Least 70 % Improvement in RA Symptomatology According to ACR Criteria

***Bmal*** - Brain and Muscle Aryl Hydrocarbon Receptor Nuclear Translocator Like-Arntl Gene

**BMAL** - Brain and Muscle Aryl Hydrocarbon Receptor Nuclear Translocator Like-Arntl Transcription Factor

**CAPRA** - Circadian Administration of Prednisone in Rheumatoid Arthritis

**CCT** - Compression Coated Tablets

**cDMARD** - Conventional Disease Modifying Antirheumatic Drug

**CFA** - Complete Freund's Adjuvant

**CIA** - Collagen-Induced Arthritis

***Clock*** - Circadian Locomotor Output Cycles Kaput Gene

**CLOCK** - Circadian Locomotor Output Cycles Kaput Transcription Factor

**COX** - Cyclooxygenase

**CRP** - C-Reactive Protein

***Cry*** - Cryptochrome Gene

**CRY** - Cryptochrome Transcription Factor

**DAS28** - Disease Activity Score with 28 Joint Counts

**DMARD** - Disease Modifying Antirheumatic Drug

**E-box** - Enhancer Box

**EULAR** - European League Against Rheumatism

**FLS** - Fibroblast-Like Synoviocytes

**GC** - Glucocorticoids

**HALO** - Hours After the Light was Turned On

**HPA** - Hypothalamic-Pituitary-Adrenal

**Ig** - Immunoglobulin

**IL** - Interleukin

**IR** - Immediate-Release

**MMP** - Matrix Metalloproteinase

**MR** - Modified-Release

**MTX** - Methotrexate

**MZR** - Mizoribine

**NICE** - National Institute for Health and Care Excellence

**NSAID** - Non-Steroidal Anti-Inflammatory Drug

**PEO** - Polyethylene Oxide

***Per*** - Period Gene

**PER** - Period Transcription Factor

**RA** - Rheumatoid Arthritis

**RANK** - Receptor Activator of Nuclear Factor-κB

**RANKL** - Receptor Activator of Nuclear Factor-κB Ligand

***Rev-Erb*** - *Rev-Erb* Nuclear Orphan Receptor

**RF** - Rheumatoid Factor

***Ror*** - Retinoid Acid-Related Orphan Receptor

**RORE** - Retinoic Acid-Related Orphan Receptor Binding Elements

**SAA** - Serum Amiloyd A

**SCN** - Suprachiasmatic Nucleus

**TAC** - Tacrolimus

**Th17** - T Helper 17 Cells

$\mathbf{T_{max}}$ - Time at which Maximum Concentration is Observed

**TNF** - Tumor Necrosis Factor

**Treg** - Regulatory T Cells

**TTFL** - Transcription-Translation Feedback Loops

**WHO** - World Health Organization

## Resumo

A artrite reumatoide (AR) é uma doença crónica autoimune que afeta articulações, músculos e tendões, e para a qual ainda não foi descoberta uma cura. Sucintamente, dois processos podem ser identificados como estando na base da fisiopatologia da AR: a resposta autoimune e a inflamação, os quais contribuem, em última instância, para a destruição articular e óssea.

A desregulação circadiana tem sido amplamente associada com a doença, onde os pacientes sofrem uma exacerbação circadiana nos seus sintomas, com rigidez, dor nas articulações e incapacidade funcional mais proeminentes no início da manhã e diminuindo ao longo do dia. Estas flutuações diárias na sintomatologia estão correlacionadas com uma disrupção na regulação circadiana de processos imunes, neuroendócrinos e inflamatórios. Consequentemente, os pacientes apresentam um aumento noturno nos níveis de hormonas pró-inflamatórias (melatonina) e citocinas e uma insuficiente produção noturna de cortisol anti-inflamatório para contrariar a cascata inflamatória da doença. Neste sentido, a sincronização da administração de fármacos com os ritmos circadianos da AR, designada cronoterapia, está a surgir como uma abordagem viável para aumentar a eficácia do tratamento e diminuir a toxicidade relacionada com o mesmo.

Deste modo, esta revisão apresenta alguns aspetos acerca da fisiopatologia da AR, da cronobiologia da doença e do valor da abordagem cronoterapêutica no seu tratamento, assim como um conjunto de evidências que suportam o potencial dos regimes de cronoterapia para as principais classes de fármacos usadas na gestão farmacológica da doença (GC, DMARDs e NSAIDs).

**Palavras-chave:** Artrite reumatoide, cronobiologia, ritmos circadianos, cronoterapia, eficácia do tratamento.

## Abstract

Rheumatoid Arthritis (RA) is a chronic autoimmune disease that affects joints, muscles and tendons, and for which a cure has not yet been discovered. Briefly, two major processes can be identified as being on the basis of RA pathophysiology: autoimmune response and inflammation, both contributing, in last instance, to cartilage and bone destruction.

Circadian dysregulation has been broadly associated with this disease, where patients usually suffer from circadian exacerbations in their symptoms, with increased stiffness, joint pain and functional disability being more prominent in the early morning and diminishing over the course of the day. These daily fluctuations in symptomatology are correlated with the disruption of circadian regulation of immune, neuroendocrine and inflammatory processes. Therefore, patients present increased nocturnal levels of proinflammatory hormones (i.e. melatonin) and cytokines and insufficient night production of anti-inflammatory cortisol to counteract the disease inflammatory cascade. In this line, the synchronization of drug administration with the circadian rhythms of RA, called chronotherapy, is emerging as a feasible approach to enhance treatment efficacy and diminish treatment-related toxicity.

Hence, this review presents some insights in RA pathophysiology, the chronobiological rhythms of the disease and the value of the chronotherapeutic approach in its treatment, as well as a body of evidence supporting the potential of chronotherapy regimens for the main classes of drugs used in the pharmacological management of the disease (GC, DMARDs and NSAIDs).

**Keywords:** Rheumatoid arthritis, chronobiology, circadian rhythms, chronotherapy, treatment efficacy.

# I. Introduction

Rheumatoid Arthritis (RA) is a chronic autoimmune disease that affects joints, muscles and tendons[1]. It is a multifactorial condition of unknown etiology characterized by an inflammatory process, which results in joint damage and functional disability[2,3]. The prevalence of this chronic rheumatic condition varies between 0.3 and 1 % being more common among the female gender and in developed countries, according to World Health Organization's (WHO) data[1].

Since it is a chronic disease, the individual and socioeconomic impacts of RA are immense. The individual burden results from the decline in physical function and quality of life, as well as the experience of extra-articular symptoms and comorbidities by some patients. Whereas the socioeconomic burden is directly associated with medical costs, along with the functional incapacity, which affects work capacity and social involvement of these patients[4]. In order to emphasize the need of an early detection, the American College of Rheumatology (ACR) and the European League Against Rheumatism (EULAR) developed classification criteria so that patients with a relatively short duration of symptoms can be identified before irreversible damages occur[5]. Additionally, in 2012, ACR published recommendations for the use of disease activity measures in clinical practice in order to achieve the same goal[6]. This way an effective treatment can be initiated before pathological changes become irreversible and the enormous impacts of the disease can be minimized.

RA has no known cure and the main goal, according to the National Institute for Health and Care Excellence (NICE) guideline for the management of RA in adults, is to achieve a target of remission or low disease activity, when the first one is not possible due to long-standing disease or additional comorbidities[7,8]. Despite the fact that the treatment of RA has dramatically changed over the past decades with the increased number of novel drugs and strategies, the therapies available are not fulfilling the needs of the patients, and the safety profile of some drugs remains a major concern[9]. Therefore, there is an urgent need to develop more efficacious, effective and secure therapeutic strategies with the aim to improve patients' quality of life. With this purpose various criteria have been developed in order to define the response of RA patients to treatment. Among them it is noteworthy the EULAR and ACR definitions which are validated improvement classifications with great relevance in the field of clinical trials[10]. The EULAR response criteria are computed using the Disease Activity Score with 28 Joint Counts (DAS28), this index combines information from swollen and tender joints, acute phase response (erythrocyte sedimentation rate (ESR)) and general health

assessment on a continuous scale ranging from 0 to 9.4. Using this scale, the patient's level of disease activity can be low (DAS28≤3.2), moderate (3.2<DAS28≤5.1) or high (DAS28>5.1) and a DAS28≤2.6 corresponds to being in remission. Through the evaluation of the individual amount of change in the DAS28, EULAR response criteria classify patients as non-responders (ΔDAS≤0.6), moderate responders (0.6<ΔDAS≤1.2) or good responders (ΔDAS>1.2)[11]. Instead of focusing in the RA state of activity, the ACR definition focuses on patient's improvement in tender and swollen joint counts, and at least three of the following variables: pain, physical function, patient's and physician's global assessment and acute-phase reactant levels (C-reactive protein (CRP) or ESR)[10,12]. According to these criteria, the minimal response required is a 20 % improvement (ACR20) in the previously referred parameters. A moderate response corresponds to a 50 % improvement (ACR50) and a 70 % improvement (ACR70) constitutes a major response to treatment[12].

Regarding the clinical presentation of the disease, it is well known that the intensity of RA symptoms is characterized by rhythmic fluctuations with joint pain and stiffness being more pronounced in the morning and diminishing over the course of the day[3]. This occurs because the pathophysiology of the disease is mainly driven by proinflammatory cytokines whose production and secretion shows a dependence on circadian clock, thus resulting in plasma levels changing over a 24-hour period[13]. In this line of thought, if we apply the knowledge we have on chronobiology and its influence on both immune system and inflammatory response we may have the potential to develop chronotherapeutic regimens to enhance the efficacy and effectiveness of the current treatment strategies available for RA[14].

## II. RA Pathophysiology: Hallmarks

RA is a common systemic inflammatory autoimmune disease that encompasses great heterogeneity in both clinical presentation and outcomes[15,16]. Given this heterogeneity, RA is considered a clinical syndrome and, although the etiology remains unknown, significant progress has been made in identifying environmental (e.g. smoking) and genetic (e.g. HLA-DRB1 risk allele) risk factors responsible for increasing disease susceptibility[15, 17].

Briefly, two major processes can be identified as being on the basis of RA pathophysiology: autoimmune response and inflammation. The role and expression of these pathological mechanisms are different in each stage of the disease, but both contribute to a common final pathway culminating in cartilage and bone destruction, characteristic of the clinical phase of RA (Figure 1).

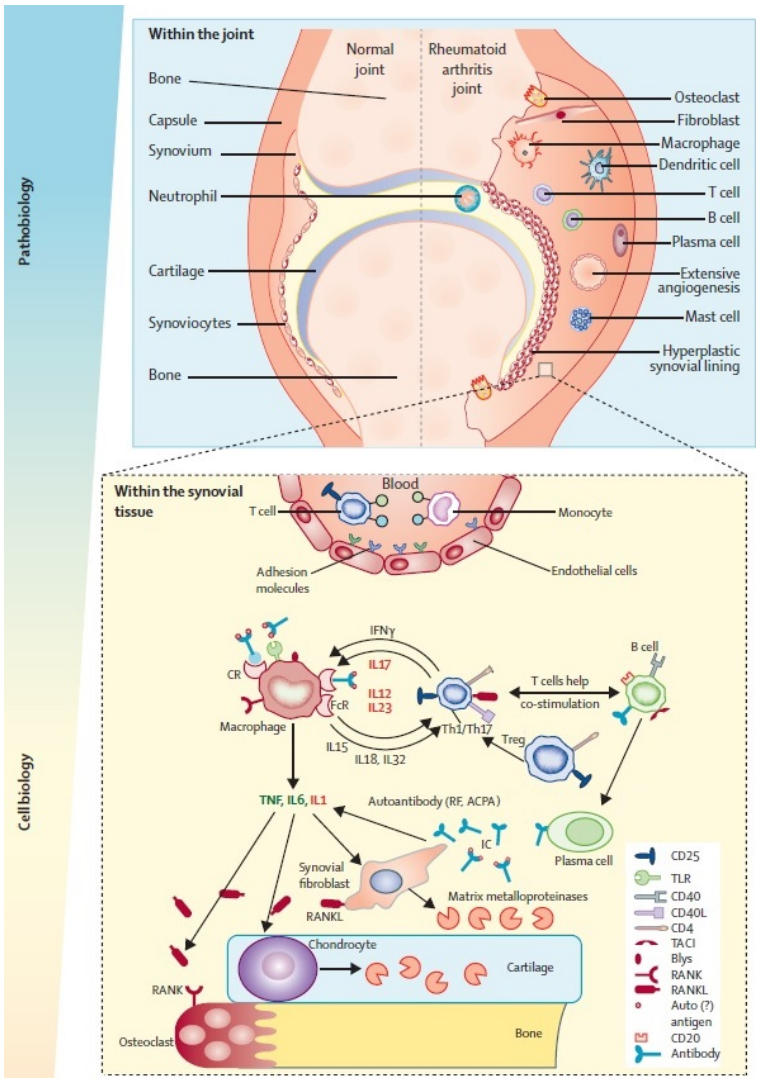


**Figure 1 - Main pathological pathways involved in RA pathophysiology[4].**
Cells from the adaptative and innate immune response together with autoantibodies play a major role in RA pathophysiology. Both contribute to a final inflammatory pathway driven by proinflammatory cytokines, thus leading to cartilage and bone damage.

In preclinical RA, the action of environmental risk factors on cells in mucosal sites seems to promote the post-translation modification of the amino acid arginine to citrulline in a diversity of self-proteins (i.e. citrullination). Following this and other post-translational modifications, the altered peptides can be recognized by the adaptative immune system thus stimulating B cells to synthesize a range of autoantibodies of which rheumatoid factor (RF) and anti-citrullinated protein antibodies (ACPAs) are the most notorious[18]. RFs are a family of autoantibodies directed to the Fc-region of Immunoglobulin (Ig) G[19]. These were the first ones discovered in RA patients and, despite their low specificity, they have been used extensively over the years to diagnose this condition[20]. Nowadays, the research on the role of autoantibodies in RA pathophysiology has been focused on ACPA headed against citrullinated proteins[17]. These are more specific and sensitive for diagnosis and seem to be better predictors of RA progression[19].

It is well known that the presence of the different autoantibodies can be detected 5 to 10 years before the actual disease onset and, depending on the stage of the disease, they can be found in 60 to 80 % of people diagnosed with RA. However, as referred, RA is pathologically heterogeneous, and this is evidenced by the existence of two types of patients: autoantibody-negative and autoantibody-positive[17, 21,22]. It is stablished that the seropositivity is associated with more severe symptoms and joint damage (i.e. increased disease activity), probably due to the formation of immune complexes by ACPA with citrulline-containing antigens and subsequent binding of RF[4,21]. As a matter of fact, in 2014 a study comparing patients with ACPA-positive and RF-negative, ACPA-negative and RF-positive, double-negative and double-positive autoantibodies was conducted by Sokoloven and his colleagues. It was observed an increased inflammation and disease activity in the double-seropositive subgroup, supporting the synergistic role of these RA-associated antibodies in the pathogenesis of the disease[23]. Furthermore, in 2015, another study showed that RF (particularly IgM isotype) is able to amplify macrophages activation and cytokine secretion, thus potentiating ACPA immune complexes' proinflammatory potential and destructive response[24].

Synovial membrane inflammation is the common final pathway experienced by RA patients. The synovium contributes to maintain cartilage homeostasis trough lubricant's production and cartilage's nutrition. The outer layer is composed of fibroblasts, scattered immune cells, blood vessels and adipocytes, while in contact with synovial fluid, there is an intimal lining composed of macrophage-like synoviocytes and fibroblast-like synoviocytes (FLS)[18]. However, because of the porous vasculature and the absence of tight junctions in the intimal lining, the ingress of cells and other components in the joint is facilitated[25].

The first main pathogenic change in RA synovium is the expansion of the intimal lining due to the increase and activation of both synoviocytes types. This activation leads to the production of a diversity of proinflammatory cytokines, such as tumour necrosis factor (TNF), interleukin(IL)-1 and IL-6, by macrophage-like synoviocytes. Despite FLS activation also leads to the expression of IL-6, its most striking feature is the production of matrix metalloproteinases (MMPs), prostaglandins and leukotrienes[18]. It is also worth mentioning that FLS show an abnormal phenotype responsible for cartilage damage[17,18]. The second change occurring in RA synovium is the infiltration of adaptative immune cells. The majority of the sublining cells are CD4+ memory T cells that can either infiltrate the tissue or differentiate and produce antibodies[18]. However, despite the adaptive counterpart of the immune response is associated with several T lymphocyte populations, one should emphasize that recent attention has been drawn towards T helper 17 cells (Th17) and regulatory T cells (Treg)[26]. Th17 cells are able to secrete proinflammatory mediators such as IL-17, which can lead to an inflammatory process through the induction of cytokines, chemokines and even mediators of bone and cartilage destruction. Regarding Treg, these are regulatory cells whose function is to suppress autoreactive lymphocytes, thus maintaining a suppressive activity in the case of RA patients[26,27]. The results of a 2015 study indicate that the development of RA is associated with a Th17/Treg imbalance in peripheral blood of patients, where there is an increase in Th17 cells prevalence and a decrease in Treg cells. Due to this disturbance, we can correlate the production of cytokines by Th17 cells with the induction of autoimmune inflammation in RA[27].

Once in synovial fluid, macrophages, neutrophils and mast cells release cytokines and MMPs thus contributing to joint damage[18]. In turn, cytokines promote chondrocyte activation leading to direct release of additional MMPs and aggrecanases to cartilage, thus promoting tissue catabolism[28,29]. Other relevant features of activated chondrocytes are that they also act as a source of proinflammatory cytokines and express several chemokines intimately involved in cell recruitment to the synovial compartment[29,30]. Regardless of its origin, the production of cytokines, that act in a paracrine or autocrine fashion, is central to enhance and preserve the inflammatory process in RA. For example, cytokines produced by macrophages can activate adjacent FLS, T cells and dendritic cells which in turn can activate other cells present in the joint[18].

In last instance, RA patients can also present bone destruction as a result from the pathological processes mentioned above. An important mechanism is related to the fact that activated fibroblasts together with T cells, B cells and macrophages can upregulate the expression of receptor activator of nuclear factor-κB (RANK) ligand (RANKL), an essential

mediator of osteoclasts formation, function and survival[4,17,31]. This ligand binds to RANK, present on macrophages, dendritic cells and preosteoclasts, triggering osteoclasts differentiation and, consequently, bone erosion via RANKL/RANK activation[4,17]. It has also been suggested that, preceding the onset of synovial inflammation, an interaction between ACPAs and citrullinated peptides expressed by osteoclasts and its precursors can lead to osteoclast maturation and activation. Therefore, ACPAs can potentially initiate articular damage[18].

## III. Chronobiology in RA: The Role of the Circadian Clock

Chronobiology is a field of biological sciences with focus on the circadian rhythms of tissue and organismal functions[32]. In order to adapt and respond to the daily environmental cycles, the organism uses internal timing systems, known as circadian clocks, that resonate with the 24-hour day cycle[33]. This circadian timing system consists in a hierarchical network encompassing a central and several self-sustainable oscillators, which drive oscillations in a diverse set of biological processes and regulate interactions between endocrine, immune, autonomic and central nervous system[2,3,34]. The majority of these transcriptional rhythms was found to be organ-specific, which indicates that, despite the molecular clock is active all over the body, it is capable to regulate biological processes quite differently in each one[34].

The mammalian circadian clock system comprehends three main components: input signalling pathways, the central clock and output signalling pathways[35] (Figure 2). At the top of this hierarchy is the central clock located in the hypothalamic suprachiasmatic nucleus (SCN), whose major function is to synchronize the peripheral clocks distributed throughout the body[33]. This master pacemaker is mainly linked to the light/dark cycle, where the photic inputs received act like a *Zeitgeber* (German word for synchronizer) being transmitted through the retinohypothalamic tract to the SCN oscillator in order to evoke time-dependent circadian responses by using circulating hormones and neural outputs[36,37,38]. Thus, SCN synchronizes ("entrains") to the light/dark cycle and, in turn, synchronizes all the other oscillators found within organs, tissues and cells[37]. Peripheral clocks employ essentially the same molecular components as the SCN and their rhythmic synchronization occurs, not only by SCN action, but also via a variety of additional factors (e.g. locomotor activity, feeding schedules and temperature), being able to function independently of the SCN[35,38,39]. Together they allow the organism to preserve the overall rhythm even following a temporary disruption on environmental signals[38,39]. In fact, in lesioned SCN models, it was found that peripheral clocks

are able to maintain some rhythmicity for a short period of time without a functional SCN or external cues[35].

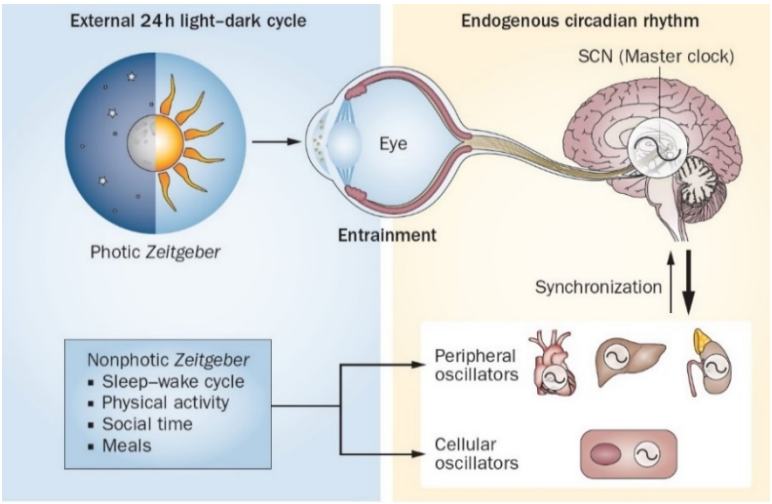


**Figure 2 - Circadian timing system[36].** The light/dark cycle is the most important Zeitgeber (synchronizer) that influences directly the master clock located in the SCN. This, in turn, synchronizes the peripheral clocks through hormonal and neural outputs. However, there are additional factors working as environmental entrainment cues like sleep-wake cycle, physical activity, social time and even meals.

The mammalian's molecular clock consists of multiple sets of transcription factors resulting in autoregulatory transcription-translation feedback loops (TTFL) operated by clock genes, encoding circadian proteins[3,39]. At the core of this network are the genes circadian locomotor output cycles kaput (*Clock*) and brain and muscle aryl hydrocarbon receptor nuclear translocator like-Arntl (*Bmal*), encoding, respectively, the transcription initiators of the feedback loops CLOCK and BMAL[40]. During daytime, the heterodimer complex CLOCK/BMAL translocates to the nucleus and binds to the Enhancer Box (E-box) of a family of Period (*Per1, Per2, Per3*) and Cryptochrome (*Cry1, Cry2*) target genes, promoting their transcription and thus leading to increases in PER and CRY levels in the late afternoon or evening. PER and CRY proteins interact with each other and, aided by post-translational modifications, enter the nucleus at night, where they are capable of interacting with CLOCK/BMAL complex in order to repress its transcription thus affecting the expression of all core-clock genes[33,40]. This feedback cycle takes approximately 24 hours, and it is the complex PER/CRY ubiquitination and subsequent degradation by the proteasome, during the night, that allows a new cycle to take place[33,38,40].

In addition to *Per* and *Cry* target genes, CLOCK/BMAL also activates retinoid acid-related orphan receptor (*Rora, Rorb, Rorc*) and *Rev-Erb* nuclear orphan receptor (*Rev-Erbα, Rev-Erbβ*), a negative feedback loop that contributes to clock's precision and robustness[33,37,40]. ROR and REV-ERB proteins have a relevant role in regulating *Bmal* transcription. ROR acts as an activator through binding REV-ERB/retinoic acid-related orphan receptor binding elements (RORE) on the promoter region of the gene. Trough competition for this binding site, REV-ERB acts as a repressor of *Bmal* expression. There are also other feedback loops involved in

the molecular mechanisms of the circadian clock, together they are able to generate cycles of transcription with different phases of expression[33]. Therefore, promoting optimal organism activity, at the same time they ensure internal homeostasis[35].

Circadian dysregulation has been broadly associated with chronic inflammatory conditions such as RA, where a striking feature can exalt the prominent role of chronobiology in the disease pathophysiology: the temporal variation in symptoms manifestation through the influence of the circadian clock[2,36]. Since RA patients suffer from chronic fatigue, immune cell dysfunction and pathogenic production of several cytokines, all of which affected by the molecular clock, Kouri *et al.* (2013) studied the hypothesis that the circadian timekeeping system is disturbed in this condition. Hence, the expression levels of clock genes in the synovial membrane of these patients were analysed through the expression of the respective inhibitory circadian genes. The antiphase expression of these inhibitory circadian components was found to be totally lost, thus demonstrating that the endogenous circadian time is clearly perturbed. However, it was also observed that inflammatory stimuli, like TNF-α, affect clock genes expression in cultured synovial fibroblasts, indicating that there is a bidirectional link between inflammation and the disturbance of the clock. In brief, it is emphasized that it is not the synovium or synovial fibroblasts that may cause these disturbances and that RA patients tend to lose the rhythm due to a cell autonomous defect in their circadian clock thus affecting the development and function of immune cells[41].

Patients with RA usually suffer from circadian exacerbations in their symptoms with increased stiffness, joint pain and functional disability being more prominent in the early morning. This fluctuation in symptoms severity is correlated with the circadian regulation of both immune and neuroendocrine systems and the expression of proinflammatory cytokines[2,14]. Since most immune cells express circadian clock genes, a wide range of immune response mediators, such as the peripheral blood mononuclear cells and macrophages, undergo daily variations in their functions[14,42]. These oscillations impact on cellular functions, just like night synthesis and release of cytokines and chemokines, and regulate circadian immune system functions, such as cell migration to inflamed tissues[43]. In fact, CD4+ T lymphocytes possess a functional circadian oscillator that drives rhythmic responses to activating stimuli thus leading to altered cell proliferation and cytokine production[44,45]. As anticipated, in this chronic inflammatory condition, patients present a nocturnal hyperactivation of the immune system functions. This is also due to the fact that energy can much easily be available during the night period, in consequence of the reduction of all other energy-consuming activities[46].

As previously referred, the endocrine system also plays an important role in mediating the propagation of circadian signals from SCN throughout the body. Two hormones with a protuberant role can be identified as circadian agents: glucocorticoids (cortisol), produced by the adrenal gland via the hypothalamic-pituitary-adrenal (HPA) axis and the autonomic nervous system, and the night neurohormone, melatonin, produced by the pineal gland[14,42]. Cortisol is considered the most potent endogenous anti-inflammatory substance, and, in contrast, melatonin is known as a proinflammatory hormone[47,48]. Both are crucial in the inflammatory pathway and act as vital regulators of immune response, with their expression following a strong diurnal pattern[14,49]. Therefore, under physiological conditions, light inputs lead to an increase in cortisol secretion and a decrease in melatonin levels, while in the absence of light stimuli (during the night) serum melatonin is generally higher[42].

In RA patients, these circadian oscillations are disrupted. Melatonin levels start to rise before the onset of clinical symptoms and before the endogenous cortisol synthesis is activated in order to counteract the inflammatory cascade characteristic of the disease pathophysiology[42]. Moreover, it is recognised that the serum levels of this hormone peak earlier than in healthy individuals and persist for a longer time, thus allowing a potentiation of the nocturnal inflammatory reaction. Consequently, the production of several cytokines, like TNF-α, IL-1 and IL-6 among others, is triggered and reach its peak during the night and early morning, shortly after melatonin serum levels are at its peak and plasma cortisol is at its lowest[47,50]. Despite the fact that proinflammatory cytokines are potent activators of the HPA axis (which results in increased cortisol), repeated elevated levels seem to lead to an adaptation of the HPA response causing a downregulation and hyposecretion of cortisol during the night period[51]. This justifies the occurrence of more severe manifestations in the early morning (Figure 3).

Despite the inflammatory role of melatonin, this hormone was also found to be able to modulate the expression of some main circadian clock genes[52]. In fact, it was demonstrated that the night neurohormone was capable to attenuate Cry1 gene expression in experimental induced mouse arthritis[50]. This result is in line with a report from another study where the absence of CRY proteins constitutively activates proinflammatory cytokine expression, particularly TNF-α and IL-6. Besides, the ablation of this core clock gene also induces a hypersensitivity of innate immune system leading to a significantly elevated secretion of proinflammatory cytokines by macrophages[53]. In addition, Bang *et al.* (2012) established that melatonin aggravates experimental induced arthritis in mice. This was supported by the

observation of pronounced infiltration of inflammatory cells and increased proliferation of synovial cells, thus stimulating the destruction of articular cartilage and bone[50].

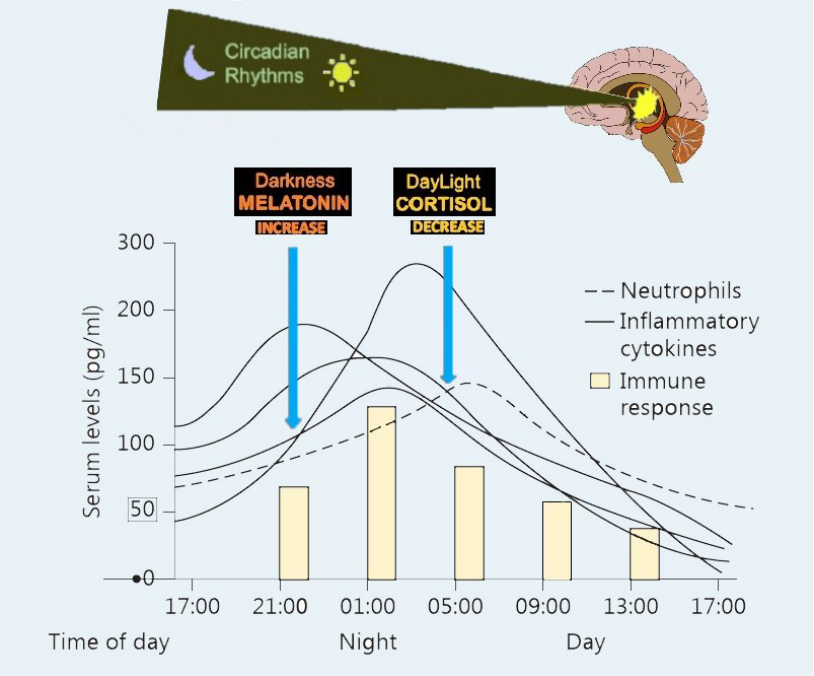


**Figure 3 - Disruption in melatonin and cortisol circadian oscillations[42].**
Cortisol is a potent anti-inflammatory endogenous substance released with light stimuli, whereas melatonin is a proinflammatory hormone released during dark periods. In RA patients, melatonin levels are higher while cortisol presents a downregulation. This disturbance leads to an exacerbation of immune response and an increased production of proinflammatory cytokines, culminating in the experience of more severe disease manifestations in the early morning.

Overall, a clear temporal relation exists between increased nocturnal levels of proinflammatory hormones and cytokines and insufficient night production of anti-inflammatory cortisol[54]. Therefore, we may conclude that morning stiffness, pain and joint swelling in patients with RA result from a hyper-inflammatory phase that induces changes in synovial fluid composition and edema of the synovial tissue and periarticular structures[42,49]. This occurs during the night and is sustained into the awakening phase due to disturbances in the normal circadian rhythms[49].

## IV. RA Current Treatment

Although there is no cure for RA, the disease modification is the mainstay of RA treatment. These modifications encompass an amalgam of purposes such as induction of disease remission, pain relief, prevention of joint and other organs damage as well as long-term complications and improvement of the patient's physical function and well-being[55]. When

remission is achieved in patients with early disease it is possible to normalize their physical function. While, in patients with established disease remission only maximizes their functional capacities. Regardless of the situation, the goal of disease remission is to prevent the occurrence of joint damage or delay its progression if the first is not possible[18].

The number of therapeutic resources available for RA treatment has grown tremendously in the past decades[8]. Currently, the pharmacological management of the disease is carried out using conventional, biologic and targeted synthetic disease modifying antirheumatic drugs (DMARDs) and glucocorticoids (GC) with the aim to reduce disease progression, while non-steroidal anti-inflammatory drugs (NSAIDs) are recommended just to ease the symptoms when control of pain or stiffness is inadequate. An outstanding strategy used is called "treat-to-target", where a treatment target (remission or low disease activity) is defined and then, a tight control is applied in order to reach and maintain that same target that can vary depending on the patient's outcomes[7]. The treatment should provide a 50 % improvement in disease activity within 3 months and the target should be achieved within the subsequent 3 months. If this is not possible, then treatment should be adapted or changed[18].

Despite some differences in the recommendations made, important similarities exist between 2018 NICE, 2016 EULAR and 2015 ACR treatment guidelines for the management of RA and all agree about the general approach to be implemented. For first-line treatment a conventional DMARD (cDMARD) monotherapy should be offered due to the disease modifying properties of these drugs[7,55,56]. Methotrexate (MTX) is the gold standard, since it presents higher clinical experience with good efficacy and safety profiles, both in monotherapy and in combination with other DMARDs. Only when MTX is contraindicated or cannot be used due to intolerance, other cDMARD, such as leflunomide or sulfasalazine, should be started. Of noting that, during the treatment it is important to define the patients' prognostic using poor prognostic markers such as moderate to high disease activity, high acute phase reactant levels, presence of autoantibodies and joint damage[55,57]. When the treatment target has not been achieved and poor prognostic markers are absent, a second cDMARD (leflunomide, sulfasalazine or hydroxycholoroquine) can be added as a step-up strategy[7,55,56]. If after that the response is still inadequate or the patient presents poor prognostic markers, a biologic agent can be recommended in monotherapy or in combination with cDMARDs[55,56]. All current biologic DMARDs target important inflammatory/immune pathways of the disease[15]. There are currently available a series of TNF-α inhibitors such as infliximab, etanercept, adalimumab, certolizumab pegol and golimumab. Although all bind TNF-α, there are significant differences in their molecular structure, administration regimens and modes of

action. To highlight that the majority of these therapeutic agents are antibodies against TNF-α, with the exception of etanercept and certolizumab pegol. The first is a recombinant fusion protein of the soluble TNF-α receptor and the Fc portion of IgG, while the second consists of a Fab fragment of a TNF antibody coupled to polyethylene glycol. Additional inflammatory/immune pathways are the target of other biologic agents such as interleukin-6 receptor antagonists (tocilizumab and sarilumab), CD80/86-CD 28 antagonist (abatacept), anti-CD 20 antibody (rituximab) and interleukin-1 antagonist (anakinra)[57]. Despite neither the EULAR nor the ACR recommendations favour the use of one specific biologic DMARD, TNF-α inhibitors are frequently prescribed as initial biologic approach (unless contraindicated)[15,55,56]. Nevertheless, the choice of the biologic agent should be made together with the patient, with consideration for his preferences in terms of route and frequency of administration and other individual factors[15]. Another option to be considered in case of inadequate response is the introduction of a targeted synthetic DMARD[55]. To date, there are two drugs approved for therapeutic use in RA, tofacitinib and baricitinib (janus-kinase inhibitors)[57].

Another point of agreement is regarding the use of glucocorticoids (e.g. prednisone, prednisolone and methylprednisolone). All treatment guidelines support that they should only be provided in short-term treatment for managing flares and rapidly decrease inflammation[7,55,56]. More often, these drugs are used in what is called "bridging treatment" where they are taken for a short period of time until the benefits of DMARD therapy arise[7,56]. GC have many well-documented adverse effects that are strongly linked to the dose and duration of the therapy, that is why they should be taken at the lowest dose possible and long-term treatment should only be considered when the long-term complications have been fully discussed, and all the other treatment options have been offered[7,15,58].

The advances in the understanding of RA pathophysiology have allowed the development of new targeted therapies to modify the natural history of the condition[9]. This way, RA has changed from a highly disabling disease, for which no effective drugs existed, to a condition that can be controlled, with many patients achieving a remission state[8]. Although the available treatment options are effective in the vast majority of patients, further studies are still needed to optimize and maximize the clinical response and prognosis[9]. Therefore, the need to explore new therapeutic agents capable of working in non-responder patients persists. For the ones whose response to therapy is still insufficient or the safety profile is a matter of concern, as well as for all other patients despite their favorable response to therapy, new treatment regimens, such as chronotherapy, could be a feasible approach as will be discussed next.

# V. Chronotherapy in RA: A Relevant Approach

As previously discussed, RA is associated with a disturbance in the molecular clock. The mechanisms underlying the disease pathophysiology present a strong temporal variation, with proinflammatory stimuli (i.e. melatonin and proinflammatory cytokines) rising during the night and early morning, while anti-inflammatory molecules (i.e. cortisol) present a downregulation. This leads to the characteristic pattern of RA symptomatology: exacerbation of stiffness, pain and joint swelling in the early hours of the day.

Given the strong circadian component of RA, where a clear temporal relation between pathologic processes and disease manifestations exists, there is an obvious potential for the use of chronotherapy regimens in disease treatment. In brief, chronotherapy is defined as the administration of medications in accordance to the biological rhythms[59]. Therefore, through the application of chronopharmacological studies to clinical treatment, we can synchronize drug delivery with the circadian rhythms of RA in order to achieve a highest efficacy and a diminished treatment-related toxicity[35].

RA chronotherapy has been extensively researched with glucocorticoids owing to the awareness that preventing the nocturnal rise of proinflammatory cytokines would be more effective than treating the established symptoms in the morning. Following the same concept, DMARDs chronotherapy regimens started to be investigated in order to improve treatment's efficacy and safety profile. In addition, and despite not being a first-line treatment option, the chronotherapeutic administration of NSAIDs to ease the symptoms has also been the goal of numerous drug development investigation teams[47].

## A. Glucocorticoids

Glucocorticoids are steroid hormones produced in the adrenal cortex. Among their different functions, the anti-inflammatory and immunosuppressive effects make them a powerful resource for RA treatment[58]. Due to their lipophilic structure, GC can easily enter the cell exerting their effects, basically on a genomic level. In the cytosol, these drugs bind to GC receptor forming a complex that subsequently translocates to the nucleus thus binding to specific DNA sequences in the promoter region of target genes. This results in either increased production of anti-inflammatory proteins (i.e. transactivation) or reduced expression of proinflammatory genes (i.e. transrepression), both processes contribute to GC effects[13,58]. In spite of being a valuable therapeutic tool for RA, these drugs present a multitude

of side effects, one of them being HPA axis suppression. This is a serious and worrying concern, classically associated with the duration and dose of the treatment (i.e. higher doses over a longer period of time cause more severe suppression). Nevertheless, further research has been suggesting that treatment regimen also influences the effects of GC therapy on HPA axis[60].

In standard regimens with immediate-release (IR) exogenous GC, these are usually administered in the morning. However, since the inflammatory cascade has already started and the morning symptoms of the disease have already been triggered, this administration schedule is considered not to be optimal. Therefore, the ideal administration of GC is during the night (i.e. before the onset of proinflammatory stimuli). In this way, since blood concentration would be very close to the normal circadian rhythms of endogenous cortisol (peaking in the early morning), the treatment efficacy would be improved[61].

In an attempt to optimize the efficacy and benefit/risk ratio of GC, a modified-release (MR) prednisone formulation has been developed for chronotherapeutic administration of the drug. Taken orally at bedtime (i.e. 10 P.M.), the tablet releases the drug approximately 4 h later (i.e. 2 A.M.) thus providing sufficient GC levels to counteract the pathophysiologic processes exacerbated during the night. The efficacy and safety of this MR prednisone was assessed and compared to IR prednisone in the Circadian Administration of Prednisone in Rheumatoid Arthritis (CAPRA-1) trial. This was a 12 week, randomised, double-blind, active-controlled study conducted in 288 RA patients with active symptoms of disease and previously receiving DMARDs and GC therapy. At the end of the treatment, the patients receiving MR prednisone at bedtime presented a substantially greater decrease in the duration of morning stiffness (primary endpoint) than the ones receiving IR prednisone in the morning. This improvement was already evident after 2 weeks of treatment and plateaued from week 7 till the end of the treatment period. A diversity of secondary efficacy outcomes, like laboratory variables, pain intensity during the day and assessment of disease activity, were also evaluated. No clinically relevant differences were noted for these variables with the exception of IL-6 levels. The MR prednisone treated group exhibited a notorious reduction in the serum concentrations of this proinflammatory cytokine (relative change of -28.6 %) while in the IR treated group these remained unchanged. Regarding the safety profile, it was comparable between the two formulations. From these findings we can conclude that the evening administration of MR prednisone, in addition to a DMARD, has a superior efficacy inducing a rapid, significant and sustained decrease in the duration of morning stiffness[61].

Following the 12-week double-blind phase, 249 patient entered a 9 months open-label extension where they were subjected to a low-dose MR prednisone chronotherapy regimen for up to 12 months. The reduction in the duration of morning stiffness and IL-6 levels observed in the MR group during the double-blind phase was sustained. Similar improvements were also evident in the patients switched from conventional IR prednisone to chronotherapy MR prednisone in the open phase. Of noting that, contrary to what happened in the double-blind study, the secondary variables pain, DAS28 and global assessment of disease activity were significantly enhanced in the chronotherapy regimen, both in the MR/MR and IR/MR treated groups[62]. Although safety and tolerability were no different from conventional prednisone, the specific impact of this new therapeutic regimen on the HPA axis was still a matter of concern. With this regard, a subgroup of 28 RA patients from the CAPRA-1 trial was subject of an additional investigation to assess the effects of long-term, low-dose prednisone chronotherapy on the HPA axis function. Corticotropin-releasing hormone stimulation tests were performed, and the result showed that there was no evidence of deterioration or onset of adrenal insufficiency, even following the 12 months chronotherapy regimen. Likewise, switching from conventional to MR prednisone did not negatively influence the HPA response. Therefore, these results clearly indicate that MR prednisone has no adverse effects on HPA axis[63]. Given the superior efficacy and safety profile reported, chronotherapy MR prednisone can thus be expected to have improved benefit/risk ratio over the IR formulation.

In the subsequent CAPRA-2 trial, performed by the same investigation team, patients with active RA and on DMARD treatment were randomized to receive MR prednisone or placebo once daily at evening for 12 weeks. In this study the primary efficacy endpoint was the proportion of patients with a 20 % improvement in RA symptomatology according to ACR criteria (i.e. ACR20 response). At week 2, the combination of chronotherapy MR prednisone and DMARDs already provided a significantly higher rate of ACR20 response than the control group (receiving placebo and DMARDs). The differences in the two groups remained significant throughout the study, with 48 % of patients receiving chronotherapy MR prednisone achieving an ACR20 response at week 12, compared to 29 % in the placebo group. DAS28 was also evaluated and, as expected, MR prednisone treatment significantly increased the proportion of patients achieving low disease activity (DAS28 ≤ 3.2). The change in the duration of morning stiffness between baseline and week 12 was the secondary endpoint. A greater reduction in the duration, severity and recurrence of morning stiffness was seen for the MR prednisone treatment regimen, with this group also presenting higher improvements in physical function and fatigue. As previously documented, this chronotherapeutic formulation,

simultaneously, allowed a greater decrease in IL-6 levels (higher anti-inflammatory potential) evidencing a superior efficacy and a good safety profile[64].

After these controlled phase III studies, Cutolo *et al.* (2013) evaluated the efficacy and safety of chronotherapy MR prednisone under conditions of daily practice. In this observational study, 950 patients treated with GC and DMARDs were switched from oral IR prednisone or 6-methyl (6M)-prednisolone to low-dose MR prednisone and followed up for 4 months. The dose reduction was only possible due to the synchronization of GC administration with the biological rhythms of RA. At baseline, the two groups presented some differences considered not statistically significant for the results. After 16 weeks of treatment, both groups displayed a significant mean improvement in all evaluated parameters (morning stiffness, pain intensity, global assessment of disease and DAS28 score). Interestingly, the difference was more pronounced in the 6M-prednisolone switched group. Since this drug is known to have a greater anti-inflammatory potential and longer biologic half-life compared to IR prednisone, one might expect a higher difference in therapeutic efficacy in the IR prednisone group contrary to what occurred. Even if we cannot be sure, the authors attributed this deviation to differences in compliance between the two formulations. Overall, these findings confirm that, even in the 6M-prednisolone subgroup, switching to chronotherapy MR prednisone can meaningfully improve RA symptoms and diminish disease activity[65].

Proven the superior efficacy of MR glucocorticoids formulations, their optimization in order to synchronize the drug release with the chronobiology of RA is one of the main objectives of the drug investigation and development field. In this line, Carciello *et al.* (2016) developed a delivery platform for prednisolone (pharmacologically active species) chronotherapy - alginate beads loaded with prednisolone in hypromellose/gellan gum capsules (DRcaps®). Therefore, the combination of the intrinsic properties of beads and capsules allowed the development of a successful chronotherapeutic delivery system capable, not only of reducing prednisolone release in simulated gastric fluid to12.6 %, but also of delaying the dissolution to about 6.5 h after pH change[66].

On the whole, there is a large body of evidence supporting the intervention with a chronotherapeutic low-dose GC regimen in established RA. MR glucocorticoid formulations demonstrated being capable of targeting nocturnal cytokine peak and, subsequently, reducing morning stiffness. Since it presents an optimized benefit/risk ratio, increased efficacy and no impact on adrenal function, we can consider this treatment option as a feasible approach to reduce the impact of RA symptomatology in patients' quality of life.

### B. Disease Modifying Antirheumatic Drugs

The interest in the chronotherapeutic approach for the use of DMARDs arises from the fact that the cells involved in the immune and inflammatory processes of RA present a circadian activation. Being these cells the preferential target of this class of drugs, its administration should be adapted to their rhythms[47].

Methotrexate is an analogue of folic acid widely used as first-line treatment for RA patients[55,56,67]. It is considered the cornerstone of therapy to which other drugs are added in order to achieve an optimal therapeutic effect[67]. The mechanism of action of MTX in RA is most likely associated with its antiproliferative and immunosuppressive effects owing to the inhibition of dihydrofolate reductase which in turn leads to an interference with purine and pyrimidine synthesis. This drug is also capable of stimulating adenosine release, thus suppressing the inflammatory functions of macrophages, monocytes, neutrophils, lymphocytes and dendritic cells involved in the pathogenesis of the disease. Despite presenting a relatively good tolerability, its toxicity is also matter of concern with leucopenia amongst the serious and most frequent observed side effects[68].

MTX dosing-time dependence efficacy and toxicity was first studied by Hideto To and his colleagues, using the collagen-induced arthritis (CIA) rodent model[69]. This represents a good disease model because of its comparability to RA in terms of pathophysiology, immunology and genetics and because of the presence of equally augmented cytokine levels[70,71]. Rats and mice were first immunized with bovine type II collagen to induce arthritis. Then, MTX (0.1 mg/kg) was orally administered to CIA rats one day after the first immunization at 10 or 22 hours after the light was turned on (HALO), every day for 3 weeks, while CIA mice received MTX (60 mg/kg) injected intraperitoneally 21 days after the first immunization at 10 or 22 HALO every 7 days for 3 weeks. The arthritis score was recorded every day and it was found to be significantly lower in the 22 HALO group than in the 10 HALO and control (receiving sodium bicarbonate) groups, both in rats and mice. These findings suggest that both the preventive and the therapeutic effects are higher in the groups treated with MTX 22 HALO. Leucopenia was also investigated in mice and no significant difference in leucocyte counts were observed between the control and treated groups. Since RA is characterized by the 24 h rhythms in cytokine levels, plasma TNF-α concentrations were measured in normal and CIA mice. In fact, both groups showed a significant 24 h rhythm with higher levels at the light phase and lower at the dark phase, still, as expected, the levels were higher at all sampling times in CIA mice. Therefore, the results show that the development of arthritis was

significantly inhibited in the 22 HALO treated group, when plasma TNF-α levels began to increase, suggesting that MTX has a significant dosing-time dependent action that may be due to the 24 h rhythms of proinflammatory cytokines[69].

Posteriorly, To *et al.* (2011) studied the dosing time-dependent effects of MTX on MRL/lpr mice, which are homozygous for the lymphoproliferation spontaneous mutation (*Fas*$^{lpr}$) and show systemic autoimmunity[72,73]. These effects were assessed through the evaluation of the 24 h rhythms in TNF-α mRNA expression and leucocyte counts and through the influence of the dosing time on Serum Amiloyd A (SAA), IgG-RF and TNF-α levels. In line with the results formerly obtained, TNF-α mRNA levels showed a clear 24 h rhythm with higher levels observed during the late dark phase, at the same time plasma SAA and TNF-α concentrations showed 24 h rhythms with peaks at 2 HALO. Among the MRL/lpr mice receiving MTX treatment 3 times a week for 2 weeks at 6 and 18 HALO, the 18 HALO group showed significantly reduced plasma SAA and TNF-α levels. In addition, both arthritis and inflammation were diminished in the dark phase (activity span of mice) at the same time that the plasma TNF-α concentrations began to increase. These results suggest that the therapeutic effects of the treatment can be improved through the administration of MTX when the proinflammatory cytokine levels begin to increase in blood[72].

Based on their findings in the RA animal model and the 24 h rhythms of RA patients, a clinical trial was subsequently performed in a sample of 22 Japanese patients already receiving MTX. The dosing schedules of the patients were changed from MTX 3 times a week (day 1: after breakfast and supper, day 2: after breakfast) to a chronotherapy regimen with the same dose and number of doses per week for each patient but administered 3 times a week at bedtime. The primary endpoints of the clinical trial were changes in the DAS28 and leucocyte counts. After 3 months of bedtime MTX chronotherapy, the DAS28 of 14 patients (in a total of 17 who received the treatment) significantly improved and the therapeutic effect of this regimen was established with 7 patients achieving a moderate response and 4 a state of clinical remission. In terms of toxicity, almost all patients showed mild leukopenia, but no severe adverse effects were observed during the trial. Regarding the secondary endpoints, these consisted of tender and swollen joint counts, patients' global assessment of disease activity, modified health assessment questionnaire and serum levels of CRP, SAA, MMP-3, RF and IL-6. Through the study there were slight changes in tender joint counts and CRP levels, however swollen joint counts markedly decreased, and patients' functional capacity was enhanced after the chronotherapeutic regimen. RA-related factors (SAA, MMP-3, RF, TNF-α and IL-6) did not

change significantly throughout the study, except SAA concentrations that decreased gradually during the 3 months and had improved by 60.6 % after chronotherapy. From this clinical trial, bedtime MTX chronotherapy shows strong therapeutic effects compared to the standard treatment, with its safety and efficacy being established through the improvements observed in patients' disease activity and functional capacity[72].

More recently, Wang *et al.* (2018) also draw attention into the potential of MTX chronotherapy. Their study to evaluate the efficacy and toxicity of this treatment regimen was performed in CIA rats. First, IL-6 serum levels were measured and, as expected, the concentration was higher in CIA rats than in normal rats. It was also observed that this proinflammatory cytokine presents a clear 24 h rhythm in CIA rats, with higher levels at 6 HALO (light phase) and lower levels at 18 HALO (dark phase). Based on these findings, MTX (1/7 mg/kg) was administered according to a chronotherapy schedule, in which one experimental group was treated at 6 HALO (when IL-6 levels started to decrease) and the other at 18 HALO (when IL-6 levels started to increase), once daily. A positive control group receiving MTX (1 mg/kg) treatment once weekly by gastric infusion (conventional therapy), and a negative control receiving phosphate buffered saline, were also part of the study. Using a scoring system, the rats were then assessed for signs of arthritis. The normal group didn't reveal any signs of the disease, with its score being zero. Between days 7 and 10 after immunization, the rats showed congestion and edema of ankle joints and, by days 10 to 16 the ankles presented swelling and hyperemia, which increased until day 21. During MTX treatment (conventional or chronotherapy) the arthritis score markedly decreased in both experimental and the control groups. However, on day 56, it was remarkably decreased in the chronotherapy experimental groups compared to the positive control (conventional therapy). Of noting that, the 18 HALO group significantly inhibited the increase of arthritis compared to the 6 HALO group. At the end of the experiment, the rats were sacrificed for histological examination of the joints. The knee joints of CIA rats displayed marked synovial hyperplasia, infiltration of inflammatory cells and partial bone destruction. In contrast, after MTX chronotherapy, the experimental groups presented histopathology scores significantly lower than those of negative control and conventional therapy groups. Moreover, it was the 18 HALO treated group which presented lower histopathology score. The effects of the treatment were also evaluated on TNF-α, IL-6 and CRP levels. These inflammatory response markers were clearly decreased in the 18 HALO experimental group compared to the controls and the 6 HALO group. To assess the safety of the treatment, leukocyte counts were performed and, despite being lower in both experimental groups, the values were found to be

within the normal range. On a whole, these results indicate that MTX chronotherapy once daily, particularly at 18 HALO, is more effective than the once weekly regimen in treating CIA rats and delaying the progression of the pathology[59].

Not only MTX, but also other drugs with disease modifying properties (e.g. tacrolimus and mizoribine) have been researched for their potential to be used in chronotherapeutic regimens.

In 2011, the dosing time-dependency of the arthritis-inhibiting effect of tacrolimus (TAC) was studied by Obayashi *et al.*[74]. TAC is an immunomodulatory drug commonly used to prevent organ transplant rejection[75]. This immunosuppressive agent acts through the formation of a complex with specific intracellular proteins that subsequently inhibit calcineurin, a calcium-dependent phosphatase required for activation of the transcription factor Nuclear Factor of Activated T cells, required for T cell proliferation and cytokine production[75,76]. Thus, its action in RA is due to its antiproliferative and anti-inflammatory effects that are also highlighted by its capacity of inhibiting prostaglandin E2 production by synovial cells and regulating MMP-13 synthesis in rheumatoid synovium[75]. A matter of concern of the treatment is TAC nephrotoxic potential, previously documented in the transplant field[74,75].

For the study conducted by Obayashi *et al.* mice were immunized with bovine type II collagen and the 24 h rhythms of SAA and cytokines were then measured. All SAA, TNF-α and IL-6 levels showed clear daily variations, with higher levels during the light phase and lower levels in the dark phase (activity span). Besides, the concentrations of these inflammatory markers were significantly higher compared to those in normal mice. According to these results, TAC (4 mg/kg) was intraperitoneally administered 17 days after the first immunization at two timing points, 2 or 14 HALO, every day for 3 weeks. From this point on, CIA mice were visually examined for the appearance of arthritis with its severity being graded on a scale based on the appearance of swelling and/or redness of the limbs. The arthritis score increased day by day after immunization, however, by day 38, the 2 HALO treated group displayed marked arthritis suppression compared with the 14 HALO experimental group and the control group (receiving saline). Despite there were no differences between the two treated groups, TNF-α and IL-6 plasma concentrations were markedly decreased with TAC administration. MRL/lpr mice were also used in this study to assess the effects of TAC on leukocyte counts, which are an inflammatory marker usually increased in most RA patients. When TAC was administered at 2 HALO there was an inhibition of the increase and a preservation of the normal leukocyte level, thus suggesting that this regimen may contribute

to the inhibition of both inflammatory response and arthritis. With nephrotoxicity being a common adverse effect of TAC, renal function was also evaluated through blood urea nitrogen, plasma creatinine and urinary N-acetyl-β-glucosaminidase activity. Since there were no significant disparities between the treated and normal group parameters, it is thought that there is no dosing-time dependent renal toxicity while using the dose that has inhibitory effects on arthritis. However, additional studies are required to access long-term safety of the treatment. Hence, the results of this study support the higher therapeutic effect of TAC chronopharmacology when administered during early morning at the same time that proinflammatory cytokine levels are increased[74].

Mizoribine (MZR) is an immunosuppressive agent that firstly demonstrated its efficacy in the renal transplantation field[77]. More recently, the clinical advantages of this imidazole nucleoside against autoimmune diseases, such as RA, has been documented[77,78]. MZR is a prodrug that, after activation, inhibits inosine monophosphate dehydrogenase (an enzyme in the pathway of nucleic acid synthesis) thus inhibiting lymphocyte proliferation[79]. Thereby, this drug presents immunosuppressive and anti-inflammatory effects that contribute to its antirheumatic action. Regarding its toxicity, MZR presents a better safety profile than other antirheumatic drugs. Conversely, its efficacy was found to be inferior, reason why it is essential to find a therapeutic regimen capable of enhancing it[78].

Kanasaki *et al.* (2012) draw attention into the role of this drug chronopharmacology in CIA rats. The investigation aimed to evaluate the dosing-time dependent antirheumatic effect based on the 24 h rhythms of CRP and TNF-α. First, TNF-α and CRP concentrations were measured, respectively, on days 14 and 20 after first immunization. Both inflammatory markers presented clear 24 h rhythms, with TNF-α levels being higher during the light phase and CRP levels peaking at the late dark phase/early light phase. Subsequently, two dosing MZR regimens were performed in the experiment. A preventive regimen with MZR (10 mg/kg) orally administered one day after first immunization and a treatment regimen with MZR (20 mg/kg) orally administered from 8 days after first immunization. Every day, the groups received the drug at 10 or 22 HALO and the arthritis score was recorded since the first immunization. In both cases the arthritis score was significantly lower in the treated groups than in the control group, with a significant suppression of arthritis on the 22 HALO treated groups. These results demonstrate a significant dosing-time dependent difference in the drug antirheumatic effect and that both preventive and therapeutic effects are higher in groups treated with MZR at 22 HALO. Regarding the most remarkable adverse effects of this drug, myelosuppression (leukopenia and erythropenia) was investigated in non-CIA rats treated with MZR. Despite

leukocyte counts were temporarily lower in the 22 HALO treated groups, the rats didn't present severe leukopenia. However, high doses of MZR (20 mg/kg) caused a decrease on erythrocyte count (i.e. dosing-time dependent erythropenia). In brief, the inhibition of the excessive increase in leukocytes and erythropenia were higher in the group treated at 22 HALO, when bone marrow cells begin to synthesise DNA. Hence, we can conclude that MZR presents dosing-time dependent antirheumatic and toxic effects, corresponding to the 24 h rhythms of cytokines and cell cycle in myelocyte cells[78].

### C. Nonsteroidal Anti-Inflammatory Drugs

Nonsteroidal anti-inflammatory drugs are commonly prescribed to RA patients in order to improve their quality of life. These drugs exert their action through the inhibition of the enzymatic activity of the cyclooxygenase (COX) enzymes (COX-1 and COX-2) involved in the synthesis of prostaglandins. They all share a common spectrum of clinical toxicities, which are a major concern in rheumatic diseases due to the higher doses required, the use of concomitant medication and patients' age[7,80]. Another main disadvantage is their short half-life which leads to the need to be administered right before the onset of the symptoms (i.e. early morning) resulting in patient's incompliance[81].

Over the past years there was an effort to develop timed-release formulations of NSAIDs so that the release of the drug can occur at a predetermined time after administration at bedtime. This way, the short half-life problem can be overcome, with the release of the drug being synchronized with the development and aggravation of RA clinical signs[82,83]. With this aim, multiple investigation teams tested and optimized several chronotherapeutic formulations of different NSAIDs. Among them, it is to emphasize those who have performed clinical/*in vivo* studies such as Sunil *et al.* (2013) with indomethacin, and Ramasamy *et al.* (2013) and Sanka *et al.* (2015) with aceclofenac[82-85].

Sunil *et al.* performed, in 2013, two studies regarding compression coated tablets (CCT) of indomethacin for chronopharmacological treatment of RA[82,83]. In one of the studies, polyethylene oxide (PEO) WSR 303 was used in the outer layer to retard the release of the drug. The resulting formulation provided a lag time of 6 h, followed by an immediate release of the drug, thus achieving the maximum concentration at around 7-8 h after administration. Clinical studies were performed in 6 healthy male volunteers divided in two groups: one received a single dose of the test chronotherapeutic formulation, and the other a single dose of the reference product (Indocap® capsule). The observed delay in the time at which

maximum concentration is observed ($T_{max}$) confirms the definite lag time of 6 h *in vivo*, which allows the release of indomethacin between 4 and 6 A.M. (if administered at bedtime – 10 P.M.) when RA symptoms are at its peak[82]. Regarding the other study from the same team, a CCT of indomethacin was developed with PEO WSR Coagulant to achieve a desired lag time of 6 h. Subsequently, this formulation pharmacokinetics was evaluated in healthy volunteers against the immediate release capsule formulation (Indocap®). Similarly to the conclusions from the previous study, the *in vivo* delayed $T_{max}$ makes this formulation useful as a chronotherapeutic drug delivery system to target the onset of RA early morning symptoms[83]. Still concerning the chronotherapeutic administration of indomethacin, Tinny *et al.* (2013) developed an osmotic device to deliver the drug after a predetermined lag time. The *in vitro* dissolution test confirmed that drug release starts after a lag time of 4 h and it is completed in 2 h, indicating that it provides sufficient plasma concentration after 6 h of sleep[86].

Also in 2013, Ramasamy *et al.* proposed a chronotherapeutic system for aceclofenac combining the pH-sensitivity property of the enteric polymer eudragit S-100 and the enzymatic biodegradability of pectin in the colon: eudragit-coated aceclofenac-loaded pectin microspheres. Since the coating of eudragit S-100 protects the drug from the acidic environment of the stomach, the expected result was its controlled release in the colonic site. Through *in vitro* studies, several formulations were evaluated, and, in all cases, the cumulative drug release was notoriously higher in the presence of rat cecal content owing to the presence of enzymes responsible for the degradation of pectin microspheres. The formulation which exhibited 80 % drug release in 8 h was selected for additional *in vivo* studies. In these, the rats were injected with complete Freund's adjuvant (CFA) to induce experimental arthritis and then subjected to treatment with standard aceclofenac or the chronotherapeutic formulation. At a dose of 10 mg/kg of body weight, both test drugs inhibited the development of swelling induced by CFA thus revealing anti-inflammatory activity. In addition, a pharmacokinetic study was performed. As expected, pure aceclofenac presented a $T_{max}$ of 0.48 h and a short half-life specifying its rapid removal from plasma. On the contrary, aceclofenac microspheres exhibited a $T_{max}$ of 8.27 h and a higher half-life which indicates that the drug remains in the body for a longer period of time, thus allowing prolonged effects. In this way, the results of the present investigation suggest that this delivery system can be effectively used for colon-specific drug release of aceclofenac, thus allowing its synchronization with the chronobiological symptoms of RA[84].

Still regarding aceclofenac chronotherapy, a pH-dependent delayed-release colon-specific microspheres formulation has been developed in 2015. In the study, the *in vivo* anti-

arthritic activity was tested in CFA induced arthritis rats. The histology of tibiotarsal joints of the animals treated with aceclofenac (pure and microspheres) presented normal bone and cartilage with mild pannus formation and no accumulation of synovial fluid. The anti-inflammatory delayed response was tested using carrageenan-induced edema in rats. Predictably, pure aceclofenac showed rapid anti-inflammatory activity, while the microspheres presented a significant anti-inflammatory activity 7 h after administration. This result indicates that this formulation shows a delayed response due to the pH-triggered property of eudragit S-100, thus preventing premature release of the drug in the upper gastrointestinal tract and facilitating the disintegration in the colon. Therefore, this chronotherapeutic formulation can release aceclofenac approximately 5 h after its oral administration, consequently allowing a bedtime ingestion to counteract the inflammatory cascade of the disease exacerbated during the night[85].

Meanwhile, several other NSAIDs chronotherapeutic formulations have been developed and studied. Lornoxicam was another NSAID targeted for a chronotherapeutic drug delivery system. Using natural polymers, Bansal & Pande (2013) formulated dual cross-linked pulsatile beads subsequently filled in an enteric-coated capsule to achieve the required lag time. Owing to the combination of different factors, the formulations prepared demonstrated a lag time of 7 to 8 h[87]. Keeping in view targeting lornoxicam during the time it is needed the most, Hadid *et al.* (2015) developed a novel pulsincap formulation using mini-tablets. In this study the desired lag time was targeted as minimum 5 h and the maximum amount of drug should be released between 6 to 8 h (i.e. the drug starts releasing at 3 A.M. and the maximum portion is available between 4 and 6 A.M.). The time-dependent release was achieved through the prevention of drug release in stomach and small intestine, and subsequent release in the colonic medium, thus allowing a chronotherapeutic administration[88].

Later in 2016, Hadi *et al.* developed a matrix-mini-tablets-filled capsule formulation in order to target naproxen to ileo-colonic junction, based on the same assumption that the administration of the formulation at 10 P.M. should allow the maximum amount of anti-inflammatory drug to be available between 4 and 6 A.M.. Despite different enzyme and pH dependent polymers were tested, the formulation encompassing eudragit S-100 was considered the best with a lag time of 2.45 ± 0.97 h. The *in vitro* release profile of this chronotherapeutic system revealed that the amount of naproxen released was approximately 27.30 %, 92.59 % and 99.38 % at the end of 5, 8 and 12 h, respectively[89]. More recently, ketoprofen and ibuprofen were the target of a chronotherapeutic drug delivery system capable of delivering the drug after a pre-determined lag time of 6 h and maintaining a constant plasma

concentration. The *in vitro* dissolution study performed confirmed the viability of the technique used (hot-melt extrusion) in delaying drug release, thus allowing the chronopharmacological treatment of RA[81].

Since all these chronotherapeutic formulations allowed to achieve the desired lag time required to relieve RA symptoms in the early morning, the potential of their suitability to be administered in RA patients at bedtime is confirmed. Thus, a higher therapeutic efficacy as well as a better compliance of the patient are expected *in vivo*.

# VI. Conclusion

Rheumatoid arthritis is a chronic autoimmune condition, which progress leads to the destruction of articular cartilage and bone erosion. An outstanding characteristic of RA patients is the early morning clinical symptoms associated with the autoimmune and inflammatory mechanisms underlying the disease pathophysiology. It is well stablished that the daily fluctuations in symptomatology are correlated with the circadian regulation of immune, neuroendocrine and inflammatory processes, which is clearly perturbed in this condition. Therefore, the night activation of both immune and inflammatory responses, along with the disruption of endocrine system oscillations lead to an overproduction of proinflammatory mediators and an insufficient release of anti-inflammatory cortisol to counteract it. Hence, preventing the nocturnal increase of proinflammatory stimuli instead of acting posteriorly to its rise is a better therapeutic strategy to relieve morning symptoms.

After decades of extensive research there is still no cure for RA and the current available treatment options are associated with several side effects and disadvantages. Harnessing the knowledge we have about how RA pathophysiology is regulated by the molecular clock, chronotherapy regimens appear as a valuable resource to improve efficacy and reduce adverse effects. The first class to be targeted for this approach was glucocorticoids, with the emergence of MR prednisone. The great results evidenced in subsequent clinical trial lead to the application of this concept to some DMARDs and NSAIDs. Numerous methotrexate studies proved its dosing-time dependence efficacy, with bedtime administration providing significant improvement in patients' functional capacity while reducing proinflammatory cytokines levels. The same night regimen was found to be valid to mizoribine treatment, while tacrolimus presented a higher therapeutic effect when administered during early morning. Regarding NSAIDs, several chronotherapeutic formulations for bedtime administration have been successfully developed with focus on overcoming the short half-life problem and, like in GC, acting prior to the night inflammatory cascade easing RA morning symptoms in a more effective way.

At the present time, the coordination of pharmacological treatment with the biological rhythms of RA pathophysiology and symptomatology presents a strong body of evidence. However, there is an evident lack of studies encompassing other pharmaceutical agents used in RA treatment. Particularly, being DMARDs the mainstay of RA management, there should be an effort to assess the chronotherapeutic potential of a greater number of drugs belonging to this class. In this line, and since there is still no information available, biologic DMARDs

should be target of intensive research to evaluate the potential of therapeutic optimization through the synchronization of its administration with the biological rhythms of the disease.

So far, the favourable results demonstrated by some drugs support the introduction of chronotherapy regimens as soon as possible so that treatments can be optimized and thus patients' quality of life can be improved. Notwithstanding these results, and since clock genes are found to be dysregulated in RA, a therapeutic strategy relying on molecules that work as activators or repressors of these can also be promising for future investigations. Moreover, different studies have demonstrated that circadian clock in different individuals entrain differently to *Zeitgebers*, resulting in different chronotypes. Such variations lead to significant variability of therapeutic responses and could be used to distinguish responder and non-responders to therapy. Hence, these findings evidence the importance of tailoring chronotherapy regimens to a single patient, pathology and drug[35].

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