Hugo Ribeiro

CONTROLAR A DOR EM DOENTES COM DEMÊNCIA AVANÇADA

O papel dos biomarcadores na caracterização da dor e a importância da farmacocinética na estratégia terapêutica para doentes em cuidados paliativos

Tese submetida à Faculdade de Medicina da Universidade do Porto para obtenção do grau de Doutor em Cuidados Paliativos

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DECLARAÇÃO

Tese de candidatura ao grau de Doutor pelo Programa Doutoral em Cuidados Paliativos, submetida à Faculdade de Medicina da Universidade do Porto e elaborada de acordo com os Critérios Curriculares desta Faculdade para Recrutamento na Carreira Académica e Admissão e Classificação de Provas Académicas, em vigor a partir de 21 de julho de 2021, nomeadamente no vertido no ponto III.2 – a respeito dos Critérios de Exigência Mínima para Submissão de Pedido de Admissão a Provas da Tese de Doutoramento.

Decorrendo do predito enquadramento, apresenta-se uma tese estruturada pela compilação de trabalhos de investigação, na forma de artigos científicos completos originais, em que o candidato é primeiro autor e que foram publicados em revistas indexadas com factor de impacto.

Todos os 6 trabalhos aqui compreendidos não foram submetidos para obtenção ou reconhecimento de outro grau nesta ou noutra instituição de ensino superior, encontrando-se reproduzidos na íntegra no corpo da tese.

Ademais, dentro do Doutoramento e durante a execução do plano de trabalho, o candidato também foi autor de várias apresentações na forma de comunicações orais. Embora estes produtos científicos não façam parte da estrutura central da tese, são também derivados do plano de trabalho realizado e têm assegurado a divulgação dos resultados em múltiplas frentes nacionais e internacionais. Uma lista abrangente destes trabalhos científicos adicionais é apresentada no currículo do candidato.

A presente tese cumpre os pressupostos do artigo 8.º do decreto-lei número 388/1970.

O candidato não escreve segundo o Acordo Ortográfico de 1990.

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LISTA DE ABREVIATURAS

ACES – Agrupamento de Centros de Saúde

AINE – Anti-inflamatório Não Esteróide

APAF-1 – *Apoptotic Protease Activating Factor 1*

ARS – Administração Regional de Saúde

BAK – *BCL2 Antagonist/Killer 1*

BAX – *BCL-2 Associated X protein apoptosis regulator*

BCL-2 – *B-cell leukemia/lymphoma 2*

CD – *Cluster of Differentiation*

CGRP – *Calcitonin Gene-Related Peptide*

CP – Cuidados Paliativos

ECSCP – Equipa Comunitária de Suporte em Cuidados Paliativos

EEG – Eletroencefalograma

FAS-L – Ligando da proteína membranar FAS (*Fas cell surface death receptor*)

GPIIb-IIIa – Glicoproteína IIb-IIIa

INFARMED - Autoridade Nacional do Medicamento e dos Produtos de Saúde

OMS – Organização Mundial de Saúde

MAC – *Membrane Attack Complex*

MeSH - *Medical Subject Headings*

NF-KB – *Nuclear Factor Kappa B*

NGF – *Nerve Growth Factor*

PAINAD - Escala de Avaliação da Dor na Demência Avançada

PET – Tomografia por Emissão de Positrões

PKA – *Protein Kinase A*

p38MAPK – p38 *Mitogen-Activated Protein Kinases*

RMN – Ressonância Magnética Nuclear

ROS – *Reactive Oxygen Species*

SIDA – Síndrome da Imunodeficiência Adquirida

TC – Tomografia Computorizada

TFG – Taxa de Filtração Glomerular

TNF-α – *Tumor Necrosis Factor alpha*

TRAIL – *TNF* (*tumor necrosis factor)-Related apoptosis-inducing ligand*

TRP – *Transient Receptor Potential*

RESUMO

Introdução:

A dor crónica é um problema de saúde pública, afetando cerca de 40% da população portuguesa. A incidência e a prevalência das demências aumentam exponencialmente com a idade, sendo que em Portugal existirão cerca de 6% de pessoas com mais de 65 anos diagnosticadas com uma demência. Mais de 50% das pessoas com demência terão dor crónica. Sendo Portugal um país envelhecido (cerca de 24% da população são idosos) e em envelhecimento (em 2050 serão mais de 34% de idosos), perspectiva-se uma dificuldade crescente na abordagem farmacológica da dor.

À medida que a demência evolui, provoca limitações crescentes, dificultando a comunicação (compreensão e expressão), afectando a capacidade do doente em expressar dor e conseguir caracterizá-la. Em doentes com demência avançada, a dor causa frequentemente *delirium*/agitação psíquica ou psicomotora, tornando a abordagem terapêutica mais difícil e complexa. Para além disto, muitas das alterações das características corporais associadas aos doentes com demência e com síndrome de fragilidade são idênticas ao envelhecimento, e acarretam importantes mudanças da farmacocinética. Desta forma, o perfil de segurança dos fármacos fica alterado e é necessária a personalização das terapêuticas de acordo com as características individuais.

Objectivos:

Esta dissertação tem o objetivo principal de contribuir para a descoberta de potenciais biomarcadores de dor, associados a plaquetas e a monócitos, no sangue periférico em doentes com demência em cuidados paliativos. É também analisada e revista a evidência científica relativamente à alteração dos perfis de segurança do paracetamol, dos anti-inflamatórios não esteróides (AINEs) e dos opióides no contexto de alterações farmacocinéticas que ocorrem com o envelhecimento, com a síndrome de fragilidade e na evolução das demências, particularmente na distribuição, no metabolismo e na eliminação dos fármacos.

Metodologia:

Os estudos laboratoriais foram observacionais, transversais, e retrospectivos sem intervenção e efectuados em células sanguíneas periféricas (plaquetas e monócitos de doentes com demência (com e sem dor) e em doentes controlo (com e sem demência). Para a identificação de biomarcadores de dor, o estudo laboratorial foi efectuado em plaquetas e monócitos recorrendo à citometria de fluxo. Nas plaquetas foi avaliada a expressão membranar absoluta e relativa de *Cluster of Differentiation* (CD) 36, CD49f, CD61, CD62p, CD59 e CD40 e nos monócitos e seus subtipos, clássicos, intermediários e não clássicos, os níveis de CD11c, CD86, CD163 e CD206. Os resultados foram correlacionados com várias variáveis incluindo idade, sexo, tipo de demência, tipo e intensidade de dor (ou dor controlada vs. não controlada através do valor da Escala de Avaliação da Dor na Demência Avançada (PAINAD) menor ou superior a 5, respectivamente), analgésicos utilizados (opióide, AINEs, paracetamol), utilizando métodos estatísticos adequados.

Foi ainda realizada uma revisão da literatura publicada nos últimos 20 anos referente à farmacocinética e farmacodinâmica relacionadas com os fármacos mais usados na abordagem terapêutica da dor, tendo como variável alterações individuais fisiológicas e fisiopatológicas que possam afetar o comportamento dos fármacos, comparando eficácia e segurança para opióides, AINEs e paracetamol.

Foi efetuada adicionalmente uma revisão narrativa sobre o papel da apoptose na dor crónica, com a identificação de várias vias de sinalização celulares que poderão conter biomarcadores de tipos de dor diferentes e potenciais novos alvos terapêuticos.

Resultados:

Relativamente ao estudo laboratorial, foram selecionados 95 doentes com doença não oncológica, e foram realizadas colheitas de sangue periférico em 53 doentes, 44 doentes com dor (38 dos quais com demência grave) e nove sem dor ou demência (controlos). A idade média dos doentes era 74,8 anos, sendo a maioria do sexo feminino (73,6%). Entre os 44 doentes com dor, 19 estavam sob tratamento com opióides (15 com demência e 4 sem demência). Dos doentes com demência, cerca de 84% tinham dor não controlada (PAINAD ≥ 5).

A percentagem de plaquetas que expressam CD36, CD49f e CD61 decresce com a presença de dor e os níveis de expressão de CD49f e CD61 reduzem significativamente quando comparados com os doentes sem dor. A percentagem de plaquetas expressando CD40 está significativamente aumentada em doentes com dor moderada a severa.

Relativamente à população monocitária, observámos nos doentes com dor um aumento significativo da subpopulação de monócitos clássicos, assim como da expressão de CD206 e uma redução do *ratio* CD163/CD206 quando se compara com os doentes sem dor. O CD11c está significativamente aumentado nos doentes com dor mista, enquanto os níveis de CD163 e CD206 estão aumentados nos doentes com dor nociceptiva, independentemente do tipo de demência apresentado pelos doentes. No entanto, os valores de CD163 estão significativamente mais baixos na presença de opióides, o que parece confirmar o papel anti-inflamatório dos opióides, através da inativação monocitária.

Para a revisão dos AINEs, foram selecionados 18 artigos, entre os quais se obteve níveis de evidência I (o naproxeno tem pior perfil de segurança renal e cardiovascular que os coxibes e que o ibuprofeno), forças de recomendação B (a acemetacina tem melhor perfil de segurança renal e cardiovascular) e forças de recomendação C (a acemetacina apresenta melhor perfil de segurança hepática).

Para a revisão dos opióides, foram selecionados 22 artigos, entre os quais se obteve forças de recomendação A (a hidromorfona, fentanilo e buprenorfina têm melhor perfil de segurança renal, não necessitando de ajuste posológico na disfuncionalidade do rim), forças de recomendação B (todos os opióides, excepto a hidromorfona e a morfina, deverão alargar a posologia nas populações com aumento do tecido adiposo) e força de recomendação C (os opióides com melhor perfil de segurança hepático são o tapentadol, a hidromorfona e a morfina).

Na revisão sobre o papel da apoptose na dor crónica, o NF-KB, as citocinas pró-inflamatórias como o TNF-α e o stresse oxidativo demonstraram estar envolvidas na modulação da apoptose e na mediação da dor neuropática periférica. A apoptose envolvida nos gânglios da raiz dorsal tem sido associada a lesões de nervos espinhais,

através da sinalização pela caspase e/ou pela ativação da PKA através da via da *p38 Mitogen-Activated Protein Kinase* (p38MAPK), originando e mantendo a dor neuropática.

Discussão e Conclusões:

As alterações corporais e de funcionalidade orgânica têm uma influência significativa na farmacocinética do paracetamol, dos AINEs e dos opióides. Os fármacos hidrossolúveis obrigam habitualmente a uma redução da dose, enquanto os fármacos lipossolúveis obrigam a um ajuste da posologia (menos administrações diárias se o doente é obeso ou idoso). Os fármacos com grande eliminação renal obrigam a ajustes posológicos e de dose. Finalmente, os fármacos com metabolização hepática na fase 1 de biotransformação devem ser substituídos sempre que possível por fármacos com metabolização hepática na fase 2 ou as posologias devem ser ajustadas para administrações mais espaçadas e com doses inferiores.

As características corporais e a funcionalidade hepática e renal são essenciais para a farmacocinética dos fármacos mais prescritos para o tratamento da dor (paracetamol, AINEs e opióides), pelo que a caracterização individual deve ser central na escolha do fármaco mais eficaz e seguro, e na dose e posologia a utilizar.

A modulação da apoptose poderá potenciar o controlo da dor crónica, com mais armas terapêuticas para novos alvos. A inibição da apoptose, através da modulação do BCL-2, da caspase 3, do TNF-α, do NF-KB, do proteasoma (utilização de inbidores do proteasoma) e do stresse oxidativo (como por exemplo os flavonóides), tem potencial para contribuir para o controlo da dor neuropática, embora ainda careça de confirmação por ensaios clínicos.

Foram identificados vários potenciais biomarcadores de dor: o aumento da percentagem de plaquetas que expressa CD62p e dos monócitos, particularmente da subpopulação de monócitos clássicos e da expressão de CD206 monocitária, bem como diminuição da percentagem de plaquetas que expressam CD36, CD49f e CD61 e dos níveis de expressão de CD49f e CD61 plaquetários poderão vir a ser considerados biomarcadores de dor.

Além disso, foram caracterizados potenciais biomarcadores periféricos da intensidade e tipo de dor, sendo de salientar o CD40 plaquetário na identificação de dor moderada a severa, e o CD62p plaquetário na identificação da dor nociceptiva. Para além disso, nos monócitos clássicos, o CD11c está significativamente aumentado nos doentes com dor mista, enquanto que os níveis de CD163 e CD206 estão aumentados nos doentes com dor nociceptiva, independentemente do tipo de demência. Estes resultados sugerem a relevância destes marcadores na caracterização da intensidade e tipo de dor e poderão contribuir para um melhor ajuste terapêutico dos adjuvantes utilizados no tratamento farmacológico da dor.

Apesar de ser o maior estudo realizado com doentes com necessidades paliativas especializadas, totalmente dependentes e próximos do fim de vida, particularmente doentes com dor e demência avançada, os dados que apresentamos nesta tese necessitam de estudos confirmativos, multicêntricos, com maiores amostras, comparando doentes saudáveis com doentes com diferentes causas de dor e diferentes mecanismos de demência, assim como diferentes respostas aos tratamentos instituídos. Será igualmente importante avaliar a farmacogenómica e o seu potencial para interferir na farmacodinâmica e na farmacocinética.

Esta tese tem a vantagem de apresentar uma visão de futuro para o tratamento da dor, apoiando a medicina de precisão, que acreditamos que reduzirá a iatrogenia, melhorando a eficácia e a segurança da farmacoterapia instituída em doentes com dor crónica.

ABSTRACT

Introduction:

Chronic pain is a public health problem, affecting around 40% of the Portuguese population. Dementia incidence and prevalence are rising, with nearly 6% of diagnosed patients in Portugal with more than 65 years. More than 50% of dementia patients will have chronic pain. As Portugal is an aged (nearly 24% are elderly) and aging country (in 2050 more than 34% of Portuguese population will be elderly), an increasing difficulty is expected in the pharmacological approach to pain.

As dementia progresses, it causes increasing limitations, making communication (understanding and expression) difficult and affecting patient's ability to express and characterize pain. In patients with advanced dementia, pain often causes delirium, making the therapeutic approach more difficult and complex. Furthermore, many of the changes in body characteristics associated with patients with dementia and frailty syndrome are identical to aging and lead to important changes in pharmacokinetics. In this way, the safety profile of drugs is altered, and it is necessary to personalize therapies according to individual characteristics.

Objectives:

This thesis aims to discover potential pain biomarkers, associated with platelets and monocytes in peripheral blood. It is also analyzed and reviewed the scientific evidence regarding changes in safety profiles of paracetamol, non-steroidal anti-inflammatory drugs (NSAIDs), and opioids in the context of pharmacokinetics changes that occur with aging, frailty syndrome, and the evolution of dementias, particularly in the distribution, metabolism, and elimination of drugs.

Methodology:

The laboratory studies were observational, cross-sectional, and retrospective without intervention and carried out on peripheral blood cells (platelets and monocytes from patients with dementia (with and without pain) and in control patients (with and without dementia).

For the identification of pain biomarkers, the laboratory study was carried out on platelets and monocytes using flow cytometry. In platelets, the absolute and relative membrane expression of CD36, CD49f, CD61, CD62p, CD59 and CD40 was evaluated and in monocytes and their subtypes, classic, intermediate and non-classical, the levels of CD11c, CD86, CD163 and CD206.

The results were correlated with several variables including age, sex, type of dementia, type and intensity of pain (or controlled vs. uncontrolled pain through Advanced Dementia Pain Assessment Scale (PAINAD) value less than or greater than 5, respectively), analgesics used (opioid, NSAIDs, paracetamol), using appropriate statistical methods.

A review of the literature published in the last 20 years was also carried out regarding pharmacokinetics and pharmacodynamics related to the most used drugs in the therapeutic approach to pain, taking as variables individual physiological and pathophysiological changes that may affect the behavior of drugs, comparing efficacy and safety for opioids, NSAIDs and paracetamol.

Additionally, a narrative review was carried out on the role of apoptosis in chronic pain, with the identification of several cellular signaling pathways that may contain biomarkers for different types of pain and potential new therapeutic targets.

Results:

Regarding the laboratory study, 95 patients with non-oncological disease were selected, and peripheral blood samples were collected from 53 patients.

The percentage of platelets expressing CD36, CD49f and CD61 decreases with the presence of pain and the expression levels of CD49f and CD61 are significantly reduced when compared to patients without pain. The percentage of platelets expressing CD40 is significantly increased in patients with moderate to severe pain.

Regarding the monocyte population, we observed in patients with pain a significant increase in the subpopulation of classic monocytes, as well as in the expression of CD206 and a reduction in the CD163/CD206 ratio when compared to patients without pain. CD11c is significantly increased in mixed pain, whereas CD163 and CD206 levels are

increased in nociceptive pain, regardless of the type of dementia presented by the patients. However, CD163 values are significantly lower in the presence of opioids, which seems to confirm the anti-inflammatory role of opioids through monocyte inactivation.

For the review of NSAIDs, 18 articles were selected, among which levels of evidence I were obtained (naproxen has a worse renal and cardiovascular safety profile than coxibs and ibuprofen), strengths of recommendation B (acemetacin has a better profile of renal and cardiovascular safety) and strengths of recommendation C (acemetacin has a better hepatic safety profile).

For the review of opioids, 22 articles were selected, among which strengths of recommendation A were obtained (hydromorphone, fentanyl and buprenorphine have a better renal safety profile, not requiring dosage adjustment in renal dysfunction), strengths of recommendation B (all opioids, except hydromorphone and morphine, should increase the dosage in populations with increased adipose tissue) and strength of recommendation C (the opioids with the best hepatic safety profile are tapentadol, hydromorphone and morphine).

In the review on the role of apoptosis in chronic pain, NF-KB, pro-inflammatory cytokines such as TNF-α and oxidative stress were shown to be involved in modulating apoptosis and mediating peripheral neuropathic pain. Apoptosis involved in dorsal root ganglia has been associated with spinal nerve injuries, through caspase signaling and/or PKA activation through the p38MAPK pathway, originating and maintaining neuropathic pain.

Discussion and Conclusions:

Body changes and organic functionality have a significant influence on the pharmacokinetics of paracetamol, NSAIDs and opioids. Water-soluble drugs usually require a dose reduction, while fat-soluble drugs require an adjustment in dosage (less frequently daily administrations if the patient is obese or elderly). Drugs with extensive renal elimination require dosage and posology adjustments. Finally, drugs with phase 1 hepatic metabolism should be replaced whenever possible by drugs with phase 2

hepatic metabolism or the dosages should be adjusted for more spaced administrations and with lower doses.

Body characteristics and liver and kidney functionality are essential for the pharmacokinetics of the most prescribed drugs for pain treatment (paracetamol, NSAIDs and opioids), so individual characterization must be central for choosing the most effective and safe drug and its dosage. Modulation of apoptosis could enhance the control of chronic pain, with more therapeutic weapons for new targets.

The inhibition of apoptosis, through the modulation of BCL-2, caspase 3, TNF-α, NF-KB, the proteasome (use of proteasome inhibitors) and oxidative stress (such as flavonoids), has potential to contribute to the control of neuropathic pain, although it still needs to be confirmed by clinical trials.

Several potential biomarkers of pain were identified: an increase in the percentage of platelets that express CD62p and of monocytes, particularly the subpopulation of classical monocytes and the expression of CD206, as well as a decrease in the percentage of platelets that express CD36, CD49f and CD61 and of platelet expression CD49f and CD61 levels could be considered pain biomarkers.

Furthermore, potential peripheral intensity and type of pain biomarkers were characterized, with emphasis on platelet CD40 for identifying moderate to severe pain, and platelet CD62p for identifying nociceptive pain. Furthermore, in classical monocytes, CD11c is significantly increased in patients with mixed pain, while CD163 and CD206 levels are increased in patients with nociceptive pain, regardless of the type of dementia. These results suggest the relevance of these markers in characterizing the intensity and type of pain and could contribute to a better therapeutic adjustment of adjuvants used in the pharmacological treatment of pain.

Despite being the largest study carried out with patients with specialized palliative needs, totally dependent and close to the end of life, particularly patients with pain and advanced dementia, the data we present in this thesis require confirmatory, multicenter studies, with larger samples, comparing healthy people with patients with different causes of pain and different mechanisms of dementia, as well as different responses to

established treatments. It will be equally important to evaluate pharmacogenomics and its potential to interfere with pharmacodynamics and pharmacokinetics.

This thesis has the advantage of presenting a vision of the future for the treatment of pain, supporting precision medicine, which we believe will reduce iatrogenesis, improving the efficacy and safety of pharmacotherapy instituted in patients with chronic pain.

INTRODUÇÃO E OBJETIVOS

O envelhecimento da população, particularmente em Portugal, tem consequências epidemiológicas assinaláveis, com repercussões nos sistemas de saúde (Veríssimo, 2014). Pelo mesmo facto, assistimos a um aumento da prevalência de doenças crónicas degenerativas, como as demências, que originam várias dificuldades ao nível dos cuidados geriátricos e paliativos (Jaul & Barron, 2017; Veríssimo, 2014).

Apesar da prevalência estimada das demências em Portugal ser conhecida (cerca de 6% em indivíduos com mais de 60 anos, ou seja, cerca de 190 mil pessoas) (Fundação Francisco Manuel dos Santos, 2023; Santana et al., 2015) a dor em doentes com demência costuma ser abordada de forma inadequada (Abreu, 2016; Achterberg et al., 2020; Batalha et al., 2012; Burks et al., 2021; Dementia Australia, 2023; Frampton, 2003; H. Ribeiro et al., 2017).

A dor crónica, definida pela existência de dor com três ou mais meses de duração (Raja et al., 2020), é um dos fatores geradores de sofrimento mais frequentes, afectando cerca 40% da população geral (Azevedo et al., 2012), cerca de 50% das pessoas idosas que vivem na comunidade e 83% das que estão institucionalizadas (Direção-Geral da Saúde, 2010). Em 2022, cerca de 24% da população portuguesa tinha 65 ou mais anos (Fundação Francisco Manuel dos Santos, 2023), sendo expectável que em 2050 tenhamos cerca de 34% de idosos (Veríssimo, 2014).

A caracterização da dor, sempre necessária, é fundamental na abordagem dos doentes com dor crónica, o que exige boas aptidões comunicacionais, além de bons conhecimentos científicos e competências clínicas. No entanto, em doentes com demência avançada, onde a comunicação está diminuída em todas as suas dimensões, não existe a possibilidade de caracterizar adequadamente a dor (Frampton, 2003). Muitas vezes, é até difícil aos profissionais de saúde aperceberem-se da existência da dor. Desta maneira, a definição de uma estratégia analgésica fica gravemente comprometida.

A Escala de Avaliação da Dor na Demência Avançada (PAINAD) é uma escala de heteroavaliação, já validada para a população Portuguesa (Batalha et al., 2012; Warden et al., 2003), permitindo atribuir a probabilidade da existência de dor em doentes com

demência avançada. Ainda assim, a incapacidade para caracterizar a dor, segundo as suas várias dimensões, particularmente a intensidade e etiologia, impede a aplicação da escada analgésica da Organização Mundial de Saúde (OMS) (Anekar & Cascella, 2023), o que dificulta a abordagem terapêutica dirigida.

A presente tese teve como objetivo principal a caracterização da dor, em doentes com demência em cuidados paliativos, e contribuir para a selecção da melhor abordagem terapêutica.

Como objectivos específicos, pretendemos:

- Identificar biomarcadores no sangue periférico que possibilitem a caracterização da dor, em intensidade e etiologia, em doentes com demência avançada, de modo a contribuir para uma melhor personalização da terapêutica e para a medicina de precisão;

- Sistematizar a abordagem das principais terapêuticas analgésicas segundo as características individuais dos doentes, tendo em conta a fisiologia do envelhecimento, a fragilidade, a polimedicação, a disfuncionalidade orgânica e outras condições que estão frequentemente presentes em doentes com necessidade de cuidados paliativos especializados;

- Contribuir para a avaliação de novos alvos terapêuticos, que acrescentam sinergismos às terapêuticas hoje existentes, reduzindo necessidade de titulação de doses, contendo os respectivos efeitos adversos, e promovendo a melhoria do estado geral dos doentes e a redução do seu sofrimento.

Esperamos conseguir definir uma estratégia terapêutica personalizada tendo em conta as características mais importantes da dor, bem como as características individuais, otimizando resultados e prevenindo efeitos adversos. Deste modo espera-se contribuir também para uma melhor qualidade de vida dos doentes.

OS BIOMARCADORES E A DOR

A dor é uma experiência multidimensional e multifactorial que pode prejudicar significativamente a qualidade de vida do doente. Uma vez que múltiplos fatores influenciam a dor e a sua caracterização depende muito do doente e da sua interpretação individual (Organização Mundial da Saúde, 2009), há uma necessidade urgente de caracterização da dor de uma forma mais objetiva através da identificação de biomarcadores por métodos não invasivos.

Um biomarcador é uma característica ou parâmetro que é observável e mensurável como um indicador de processos biológicos normais, processos patológicos ou de respostas a uma exposição ou intervenção, incluindo intervenções terapêuticas (FDA-NIH Biomarker Working Group - Food and Drug Administration (US), 2016). Pode apresentar-se como uma característica fisiológica, molecular, histológica ou imagiológica, entre outras.

A identificação e doseamento de um biomarcador do sangue periférico implica uma técnica de colheita de sangue, minimamente invasiva (Lin et al., 2021), geralmente por punção da veia cubital mediana, da veia cefálica, ou da veia basílica do membro superior (Liu, 2020).

Têm sido publicados alguns estudos que relacionam biomarcadores com dor, com o objetivo primário de a identificar e caracterizar o mais precocemente possível, existindo ainda o objetivo secundário de definir estratégias terapêuticas direcionadas (Davis et al., 2020; Niculescu et al., 2019; Reckziegel et al., 2019; Tonelli Enrico et al., 2022) e monitorizar a resposta às terapêuticas e estudar novos fármacos.

A descoberta e o desenvolvimento de biomarcadores da dor deverá começar com o autorrelato do doente e a identificação de uma série de sinais e sintomas, associando-os a processos fisiológicos, fisiopatológicos e etiológicos da dor. Depois de obtidos, os biomarcadores poderão ser utilizados para a identificação da predisposição para a dor e dos mecanismos, para a caracterização da dor, e para o seguimento e avaliação da resposta ao tratamento (sucesso ou falha terapêutica), como está representado na Figura 1.

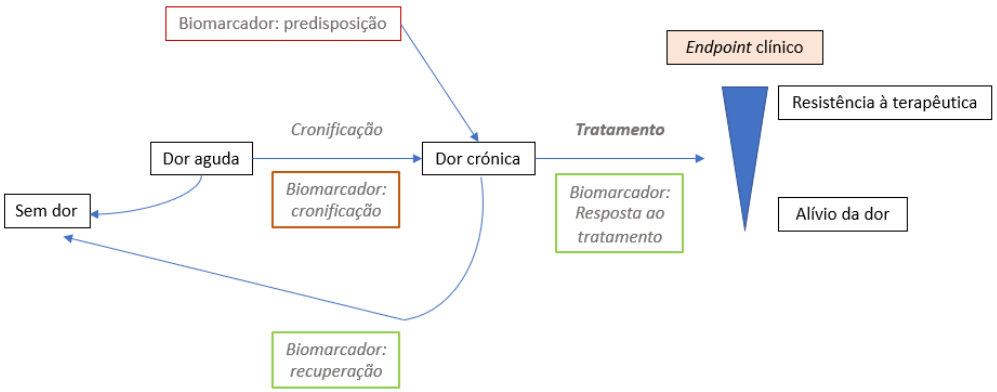

Figura 1: Utilidade dos biomarcadores da dor (adaptado de Davis et al., 2020)

Perante um doente que refere dor aguda, é fundamental avaliar, no caso da dor não desaparecer, se esta evolui para dor crónica com necessidade de tratamento adequado. O objectivo é que este tratamento permita o alívio da dor, sem o desenvolvimento de resistência à terapêutica. Em todo este processo é fundamental encontrar biomarcadores que permitam avaliar a predisposição para dor crónica (biomarcadores de predisposição ou risco), os mecanismos de dor e sua estratificação diagnóstica e prognóstica (evolução para dor crónica-cronificação/cronicidade – biomarcadores de diagnóstico/prognóstico/cronicidade), e de resposta ao tratamento (biomarcadores de resposta – recuperação ou resistência)

O processo para a validação de biomarcadores que permita posteriormente o seu uso clínico implica, como já foi dito, primeiramente a identificação e quantificação do biomarcador. Seguidamente, deverão fazer-se ensaios que avaliem e atestem a sua validade no que se refere à identificação da condição/doença, ao prognóstico, como alvo terapêutico e resposta à intervenção, prevenindo enviesamentos. Para este processo é igualmente fundamental confirmar a reprodutibilidade, a precisão, a estabilidade e a flexibilidade (Davis et al., 2020), como se visualiza na Figura 2.

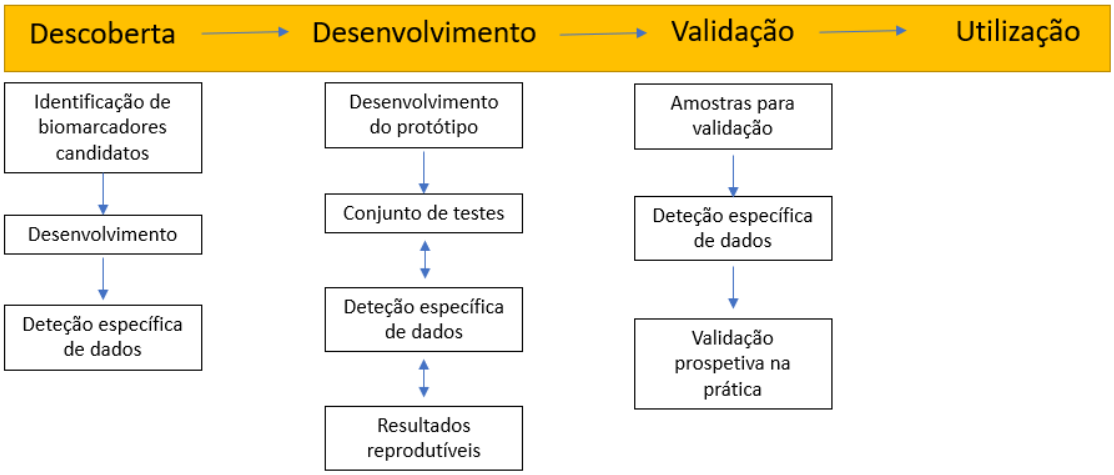

Figura 2: Passos para a identificação e desenvolvimento de biomarcadores para utilização clínica (adaptado de Davis et al., 2020)

Apesar dos múltiplos esforços, ainda não foi possível identificar biomarcadores robustos de dor. Alguns dos que estão em estudo estão representados no quadro 1 (Davis et al., 2020).

Quadro 1: Alguns dos principais biomarcadores para a dor em estudo (adaptado de Davis et al., 2020)

Biomarcador	Técnica	Possibilidade de utilização
Microneurografia (Ackerley & Watkins, 2018; Serra et al., 2012)	Registo de potenciais de ação espontâneos em fibras nervosas nociceptivas	Em investigação
Eletrofisiologia (Zurier, 2018)	Neurónios nocicetivos induzidos derivados de *stem cells* pluripotentes e biópsias tecidulares	Em investigação
Electroencefalograma (EEG) (LeBlanc et al., 2016; Levitt & Saab, 2019; Pinheiro et al., 2016; Ploner & May, 2018; van der Miesen et al., 2019)	*Laser* e potenciais evocados Espectro de frequência temporal Coerência Conectividade efetiva	Disponível e calibrado ou em calibração
Calcitonine Gene-Related Peptide (CGRP) (Goadsby et al., 2017a; Scuteri et al., 2019)	Bioquímica	Apenas em laboratórios especializados
Biópsia tecidular (Smith et al., 2017; Vlčková-Moravcová et al., 2008)	Densidade de fibras nervosas epidérmicas	Apenas em laboratórios especializados
Microscopia da córnea	Terminais de fibras nervosas na camada córnea	Equipamento disponível clínicas especializadas e oftalmologistas

Genoma (Dorsey et al., 2019; LaPaglia et al., 2018; Ramsden et al., 2017; Tracey et al., 2019; Zorina-Lichtenwalter et al., 2016)	Genotipagem e sequenciação	Disponível e calibrado ou em calibração
Epigenoma (Douglas et al., 2015; López-González et al., 2017; Ramanathan & Ajit, 2016)	Metilação do ADN e matrizes de microARN	Apenas em laboratórios especializados, ainda não calibrado
Transcriptoma (LaPaglia et al., 2018; Ramsden et al., 2017)	Sequenciação de ARN	Disponível e calibrado ou em calibração
Imunoma (Gaudillière et al., 2014; Ji et al., 2016; Raoof et al., 2018; Tsai et al., 2019)	Citometria de fluxo Parâmetros de estimulação de células mononucleares no sangue periférico	Disponível e calibrado ou em calibração
Imagem estrutural (Ressonância magnética (RMN), Tomografia computorizada (TC)) (Bosma et al., 2018; Goadsby et al., 2017b; Jones et al., 2004; Kutch et al., 2017; Staikopoulos et al., 2016; Wager et al., 2013)	Análise espectral da pele e sangue	Disponível e calibrado ou em calibração
Tomografia por Emissão de Positrões (PET) (Bosma et al., 2018; Goadsby et al., 2017b; Jones et al., 2004; Kutch et al., 2017; Staikopoulos et al., 2016; Wager et al., 2013)	Fluxo sanguíneo Metabolismo de neurotransmissores Biomarcadores da microglia	Em investigação
Testes sensitivos quantitativos (Ashraf et al., 2009; LaChapelle et al., 1999; Sikka et al., 2015)	Deteção de parâmetros de sensibilidade a estímulos nociceptivos e não nociceptivos Modulação da dor Alterações da dor com o tempo Alterações de músculos faciais relacionados com dor	Disponível e calibrado ou em calibração
Movimento e atividade (Gholami et al., 2010; M. Yang et al., 2012)	Dispositivos médicos não invasivos	Disponível e calibrado ou em calibração
Resposta autónoma (Geuter et al., 2014; Smith et al., 2017)	Condutância cutânea, diâmetro pupilar e indicadores fisiológicos de atividade autonómica	Disponível e calibrado ou em calibração

No contexto da abordagem terapêutica da dor, os marcadores biológicos (*omic-based*) poderão ser extremamente relevantes não só na identificação e caracterização da dor, dos seus mecanismos fisiopatológicos mas também na individualização terapêutica (Mamas et al., 2011).

Alguns estudos têm demonstrado que a dor nociceptiva e a dor neuropática têm associados diferentes biomarcadores (Marchi et al., 2009). Todavia ainda não foi

possível relacionar nenhum biomarcador com a duração da dor e com a sua intensidade, mantendo-se estas e outras características da dor ainda dependentes do autorrelato dos doentes (Ribeiro et al., 2017).

Embora alguns modelos pré-clínicos de dor crónica mimetizem o que se passa na prática clínica, poucos biomarcadores têm tido ampla utilidade na prática clínica (Sam Eldabe et al., 2022). A identificação e doseamento de biomarcadores por técnicas minimamente invasivas, pode ser extremamente útil em doentes com demência. As células sanguíneas, nomeadamente as plaquetas e os monócitos, devido à sua participação na resposta inflamatória e imune, poderão apresentar relevantes biomarcadores para caracterização e monitorização da dor de uma forma regular.

AS PLAQUETAS E A DOR

As plaquetas têm um papel fundamental comprovado na hemostase, na trombose e na cicatrização. Além disso, tem vindo a ser avançada a sua importante atuação na reação inflamatória e imune (Lievens & Hundelshausen, 2011; Thomas & Storey, 2015; von Hundelshausen & Weber, 2007).

Já é reconhecido o potencial papel das plaquetas para a nocicepção e a dor, uma vez que nos seus grânulos armazenam e libertam substâncias alogénicas como a serotonina, a histamina e a bradicinina (Arman et al., 2015; Ringkamp et al., 1994).

Os antiagregantes plaquetários inibem a dor (Barkai et al., 2019; Kwon et al., 2020; Salem, 1980; Weth et al., 2015), pelo que, em doentes com dor, a avaliação da atividade plaquetária poderá ser um passo importante para a identificação e caracterização da dor, o que poderá beneficiar um *follow-up* clínico mais objetivo (Gianazza et al., 2020; Ludwig et al., 2022).

As funções plaquetárias de reconhecimento, adesão, agregação, ativação, inflamação e modulação da resposta imune, apresentam várias moléculas, receptores e vias de sinalização celular que podem ser utilizadas como marcadores da dor (Gianazza et al., 2020; Ludwig et al., 2022). Alguns receptores da membrana plaquetária, como o CD62p (P-selectina) e o CD41 (GPIIb-IIIa) têm vindo a ser identificados como marcadores da

ativação das plaquetas (Akingbade et al., 2018). Neste contexto, na investigação desta tese foram utilizados como biomarcadores a glicoproteína IV (CD36), as moléculas de adesão, a integrina α6 (CD49f), a integrina β3 (CD61) e a P-selectina (CD62p), a proteína de ativação do complemento (CD59) e o receptor da família do *Tumor Necrosis Factor alpha* (TNF-α) (CD40). O CD36 é um receptor da família *scavenger* classe B, que reconhece e interage com vários ligandos e está envolvido na hemostase, trombose, malária, inflamação, metabolismo lipídico e aterogénese (Daviet & McGregor, 1997). O CD49f é uma molécula de adesão, que tem vindo a ser associada à inflamação, através da regulação da adesão e da diferenciação das células pluripotentes (Barbar et al., 2020; Z. Yang et al., 2015). O CD61 é uma glicoproteína com um papel relevante na agregação plaquetária, sendo igualmente um receptor para fibrogénio, fibronectina, factor de von Willebrand e vitronectrina (Heemskerk et al., 2013). O CD62p é uma proteína membranar das plaquetas envolvida na ativação e degranulação plaquetária e medeia a interação entre as plaquetas e as células endoteliais com os leucócitos (Hegazy et al., 2021; Kappelmayer & Nagy, 2017). O CD59 é uma proteína inibidora da ativação do complemento, que impede a polimerização de C9, impedindo a formação do *membrane attack complex* (MAC) (Weinstock et al., 2015; Xie et al., 2020). O CD40 é um receptor da família TNFα, uma citocina produzida por múltiplas células, com efeitos citotóxicos, envolvida na apoptose, inflamação, proliferação celular e diferenciação (Dostert et al., 2019).

Recorrendo a estes potenciais biomarcadores plaquetários, pretendemos avaliar de que forma poderão estar relacionados com a dor (através do aumento ou redução da sua concentração e/ou quantidade), e se estão associados a diferentes tipos de dor (dor nociceptiva, dor neuropática ou dor mista) e de diferentes intensidades (dor ligeira ou moderada-severa), avaliando a possibilidade de definição de *cut-offs* para estes efeitos.

OS MONÓCITOS E A DOR

Os monócitos são células envolvidas na promoção e na resolução de processos inflamatórios (Charach et al., 2019). Estão descritos 3 subtipos de monócitos, os clássicos (aproximadamente 85%), os não clássicos (aproximadamente 10%) e os

intermediários (aproximadamente 5%), que têm diferenças de expressão de CD14 e de CD16 (Kapellos et al., 2019; Marimuthu et al., 2018). Estes subtipos têm diferentes papéis na homeostasia, na inflamação e em várias doenças causadoras de dor, como a artrite reumatóide, a síndrome dolorosa regional complexa, as neoplasias malignas, a síndrome da imunodeficiência adquirida (SIDA) e a tuberculose (Kapellos et al., 2019; Oggero et al., 2022; Ritz et al., 2011; Tippett et al., 2011). O CD14 é uma glicoproteína membranar dos monócitos, macrófagos e alguns granulócitos, embora também possa circular livre no espaço extracelular, tendo como função a ativação celular após reconhecimento de padrões moleculares associados a patogénios (Zanoni & Granucci, 2013; Ziegler-Heitbrock & Ulevitch, 1993). O CD16 é uma proteína membranar encontrada nos monócitos, macrófagos, neutrófilos, células NK e alguns linfócitos T, envolvida na ativação da desgranulação, fagocitose e stresse oxidativo (Aguilar et al., 2022; Georg et al., 2022).

Alguns estudos têm demonstrado um aumento significativo dos monócitos intermediários em doenças inflamatórias (Kapellos et al., 2019; Marimuthu et al., 2018) e dos monócitos totais em várias patologias cujo principal sintoma é a dor (Goehler et al., 2014; Ożańska et al., 2020; Taylor et al., 2016). Para além disso, os monócitos apresentam várias moléculas, receptores e vias de sinalização celular que podem ser utilizadas como marcadores da dor.

A presente investigação avaliou o impacto da dor nos valores de monócitos totais e nas populações de subtipos de monócitos, no sangue periférico, e nas proteínas transmembranares CD11c, CD86, CD163 e CD206, que estão relacionados com várias condições, incluindo a resposta imunitária, a inflamação e a resposta à infeção (Auffray et al., 2009; Espinoza & Emmady, n.d.; Moura, 2011; Santoni et al., 2015). O CD11c é uma proteína da cadeia α da integrina, com afinidade para fragmentos do complemento, fibrinogénio e moléculas de adesão celular, envolvida na fagocitose, quimiotaxia e produção de citocinas pelos monócitos/macrófagos, assim como na proliferação de Linfócitos T (Sadhu et al., 2007; Stuhlmüller et al., 2010; Wu et al., 2018). O CD86 é uma proteína transmembranar expressa em células dendríticas, células de Langerhans, macrófagos e células B, envolvida na ativação de linfócitos T e na produção de citocinas (Halliday et al., 2020; Sansom et al., 2003). O CD163 é uma proteína com

grande afinidade para o complexo hemoglobina-haptoglobina e, na ausência de haptoglobina, apenas para a hemoglobina, envolvido na resposta imune e inflamatória, estando aumentada em doenças como a artrite reumatóide, cirrose hepática, diabetes tipo 2, linfoma de Hodgkin e várias infeções (Kowal et al., 2011; Pey et al., 2014; Roberts et al., 2004; Tippett et al., 2011). O CD206 é uma proteína membranar dependente de cálcio, presente nos macrófagos, nas células dendríticas imaturas, em células endoteliais do fígado e em fibroblastos e queratinócitos da pele, envolvida na resposta imune inata e adaptativa, nomeadamente na fagocitose de patógenos, apresentação de antigénios ao complexo major de histocompatibilidade, na produção de citocinas, na ativação e libertação de hidrolases lisossomais anti-inflamatórias e de plasminogénio, na ativação de neutrófilos e libertação de mieloperoxidase, cujo objetivo principal é o controlo das infeções e a contenção da inflamação (Holder et al., 2014; Tanaka et al., 2021; Tsuchiya et al., 2019).

Com estes potenciais biomarcadores monocitários, pretendemos avaliar de que forma estão relacionados com a dor (através da variação da sua concentração), e se estão associados a diferentes tipos de dor (dor nociceptiva, dor neuropática ou dor mista) e a diferentes intensidades de dor (ligeira ou moderada-severa), avaliando a possibilidade de se apresentarem *cut-offs* para a melhor caracterização da dor.

O PAPEL DA FARMACOCINÉTICA NA ABORDAGEM INDIVIDUALIZADA DA DOR

A farmacocinética determina o início, a intensidade e a duração de ação de um fármaco (Lötsch, 2005; Ribeiro et al., 2017; Veríssimo, 2014). Ou seja, enquanto a farmacodinâmica se refere aos mecanismos de ação dos medicamentos, a farmacocinética relaciona-se com o seu perfil de segurança (Katzung et al., 2014; H. Ribeiro et al., 2017), incluindo a absorção, a distribuição, o metabolismo e a eliminação dos fármacos.

Os doentes em cuidados paliativos apresentam habitualmente alterações na sua composição corporal, incluindo alterações da água, da gordura, e também alterações da funcionalidade hepática e da funcionalidade renal (Franken et al., 2016; Gaertner et al., 2012; Riechelmann et al., 2008; Scotton et al., 2019), que no caso de doentes idosos

poderão ser ainda mais marcadas, atendendo à fisiologia do envelhecimento (Katzung et al., 2014; Veríssimo, 2014). Todas estas alterações, assim como a maior probabilidade de interações farmacológicas pela polimedicação mais frequente (Bowie & Slattum, 2007; Bruno et al., 2014; Forrest et al., 1982; Overholser & Foster, 2011; H. Ribeiro et al., 2017; Veríssimo, 2014), devem obrigar a uma reflexão sobre ajustes posológicos e de doses a administrar aos doentes, atendendo às suas características individuais.

Assim sendo, de acordo com a fórmula de cálculo para o volume de distribuição dos fármacos (volume de distribuição = quantidade / concentração do medicamento) (Katzung et al., 2014), e tendo em conta que os doentes em cuidados paliativos poderão ter menos 20 a 40% de água corporal (Freire, 2021; Ribeiro et al., 2017; Veríssimo, 2014), é importante determinar até que ponto serão necessários ajustes posológicos, de doses e de titulações adequadas a cada doente, de forma a promover o benefício dos medicamentos e atenuar ou limitar os seus efeitos adversos. Por outro lado, em doentes obesos, e durante o envelhecimento, há um aumento do tecido adiposo, sendo igualmente importante determinar se são necessários ajustes posológicos e de dose relacionados com esta alteração do biótipo, nomeadamente para fármacos lipossolúveis.

Relativamente à funcionalidade hepática, reconhece-se que existem duas fases de biotransformação hepática: fase 1, com as reações de oxidação (onde está presente o complexo enzimático do citocromo P450), redução e hidrólise e fase 2, com as reações de conjugação e glucuronidação (Almazroo et al., 2017; Katzung et al., 2014). Cerca de 80% dos fármacos são metabolizados por vias do citocromo P450, ou seja, pela fase 1 de biotransformação hepática (Zhao et al., 2021). Uma das características do envelhecimento é a redução de cerca de 50%, dos 30 aos 70 anos, na atividade enzimática da fase 1 (Katzung et al., 2014; Ribeiro et al., 2017; Zhao et al., 2021), ao contrário da estabilidade fisiológica que ocorre com os processos da fase 2. Atendendo à fragilidade dos doentes em cuidados paliativos, e à polimedicação frequente, é fundamental determinar que alterações no raciocínio e decisão clínica deverão existir para a escolha dos medicamentos, das suas doses, posologias e titulações a aconselhar perante as condicionantes atrás descritas.

Quanto à funcionalidade renal, é reconhecida a diminuição fisiológica da função renal que ocorre com o envelhecimento (redução de cerca de 0,75 mililitros por minuto por ano, a partir dos 40 anos), sendo que cerca de 70% dos idosos com 75 anos terão menos de 50% da taxa de filtração glomerular que teriam quando tinham 40 anos (Katzung et al., 2014; Ribeiro et al., 2017; Veríssimo, 2014). Para além disso, mais de 90% dos fármacos são eliminados pela urina (Lea-Henry et al., 2018), o que significa que é importante determinar as alterações de doses, posologias e titulações que terão de ser consideradas para os fármacos tendo em conta esta característica.

A APOPTOSE E AS POTENCIAIS NOVAS ARMAS TERAPÊUTICAS PARA A DOR

A apoptose é a morte celular programada, na qual estão envolvidos vários mecanismos bioquímicos e características morfológicas (Elmore, 2007; Häcker, 2000). Vários destes mecanismos estão também relacionados com a dor crónica, particularmente com a dor neuropática (Teixeira-Santos et al., 2020; van Hecke et al., 2014).

A apoptose tem um papel vital na resposta à lesão de tecidos, à inflamação e à neuroinflamação, e nos processos de reparação e de regeneração de tecidos (Arienti et al., 2019; Y. Yang et al., 2015). Intervêm na apoptose várias vias de sinalização celular, intrínsecas e extrínsecas. A via extrínseca inicia-se pela ativação de receptores da família do TNF, através do FAS-L, TRAIL e NGF, que por sua vez ativam uma cascata de caspases (caspase 8 e 3). No caso da via intrínseca, mediada pelas mitocôndrias, são libertadas proteínas da família BCL-2 e citocromo c, que se ligam ao APAF-1 e caspase 9, formando um complexo (apoptosoma), que recruta as caspases 3 e 7, induzindo a apoptose (Du et al., 2000; Green, 2000; Verhagen et al., 2000). O stresse oxidativo pode ativar a proteína p53, que por sua vez ativa proteínas proapoptóticas da família BCL-2, como as proteínas BAX e BAK. Para além disso, o stresse oxidativo pode induzir apoptose neuronal ativando a proteína BAX através da PKA e p38 (Deng et al., 2020; Duitama et al., 2020; B. Li & Dou, 2000).

Todos estes processos estão relacionados com a inflamação e dor crónica, particularmente com a dor neuropática, havendo uma investigação acentuada para encontrar biomarcadores de dor relacionados com os mecanismos da apoptose e,

também, para procurar descobrir novos alvos terapêuticos e produzir novas armas terapêuticas (Bittar et al., 2017; Ge et al., 2014; Gloire et al., 2006; Sandoval et al., 2018; Yowtak et al., 2013).

MATERIAL E MÉTODOS

Para os artigos de revisão narrativa sobre os opióides e sobre os AINEs, foram realizadas pesquisas bibliográficas nas bases de dados PubMed/MEDLINE, *Database of Abstracts of Reviews of Effects, The Cochrane Library*, *National Guideline Clearinghouse*, *National Health Service Evidence* e Index das Revistas Médicas Portuguesas. Relativamente ao artigo sobre os opióides, para a seleção dos artigos e outros documentos foram utilizadas equações com os seguintes termos Medical Subject Headings (MeSH): *Analgesics, Opioid* and *Pharmacology, Clinical*. Foram também utilizados os resumos das características do medicamento de todos os opióides disponíveis em Portugal, através de pesquisa na página eletrónica da Autoridade Nacional do Medicamento e dos Produtos de Saúde (INFARMED), assim como as características farmacocinéticas e farmacodinâmicas destes medicamentos registadas no DrugBank®. Relativamente ao artigo sobre os AINEs, para a seleção dos artigos e outros documentos foram utilizadas equações com os seguintes termos MeSH: *pharmacokinetics* sequencialmente e juntamente com alguns dos AINEs mais prescritos a nível mundial, *ibuprofen*, *diclofenac*, *acemetacin*, *naproxen*, *etodolac* e *etoricoxib*. Foram também utilizados os resumos das características do medicamento de todos os AINEs disponíveis em Portugal, através de pesquisa na página eletrónica da Autoridade Nacional do Medicamento e dos Produtos de Saúde (INFARMED), assim como as características farmacocinéticas e farmacodinâmicas destes medicamentos registadas no DrugBank® e no Pharmgkb®.

Para o artigo de revisão sobre a apoptose, foi realizada uma pesquisa bibliográfica nas bases de dados PubMed/MEDLINE, *Database of Abstracts of Reviews of Effects, The Cochrane Library*, *National Guideline Clearinghouse*, *National Health Service Evidence* e Index das Revistas Médicas Portuguesas, tendo sido escolhidos os termos MeSH *pain* and *apoptosis*.

Os estudos laboratoriais foram observacionais, transversais, retrospetivos sem intervenção. A amostra foi seleccionada de uma população de 95 doentes com patologia não oncológica seguidos por uma Equipa Comunitária de Suporte em Cuidados Paliativos da região Norte de Portugal, entre 1 de setembro de 2021 e 1 de junho de 2022. Em cinco doentes os seus representantes legais recusaram a

participação no estudo, 20 doentes estavam em situação de últimos dias de vida na data agendada para a colheita de sangue, pelo que foi decidido não a efetivar. Por razões éticas, em 17 doentes, dado o seu estado de fragilidade, hipovolémia e maus acessos venosos, não foi possível efetuar uma colheita adequada. Assim, para os estudos laboratoriais, a amostra foi constituída por 53 doentes, a quem foram realizadas colheitas de sangue periférico depois de se terem verificado todos os critérios de inclusão e exclusão.

INSTRUMENTOS DE RECOLHA DE DADOS – ESTUDOS LABORATORIAIS

A colheita de dados dos doentes utilizados nos estudos laboratoriais foi feita através de registo em folha de *Excel*® devidamente protegida, não sendo transferida a identidade do doente, sendo este identificado pelo número de doente do estudo, que será eliminado *a posteriori* de acordo com o Regulamento Geral de Proteção de Dados (Parlamento Europeu e o Conselho da UE, 2016). As variáveis constantes nessa base de dados são apresentadas no Anexo 1. Os questionários/instrumentos de medição a aplicar presencialmente encontram-se disponíveis no Anexo 2. O modelo do consentimento informado encontra-se no Anexo 3.

Os estudos foram aprovados pela Comissão de Ética da Faculdade de Medicina da Universidade do Porto, no dia 21 de Outubro de 2021 (parecer 13/CEFMUP/2021) e pela Comissão de Ética da Administração Regional de Saúde (ARS) do Norte, no dia 13 de Janeiro de 2022 (referência CE/2022/5).

VISÃO GERAL DA TESE - ARTIGOS PUBLICADOS

Os doentes com demência avançada têm um estado de dependência e fragilidade severas, habitualmente com défices funcionais acentuados, motores, cognitivos, mentais, nutricionais e orgânicos. A abordagem farmacológica da dor nestes doentes é ainda mais difícil pelo facto de o autorrelato estar seriamente comprometido.

Neste contexto, a presente tese começou por abordar as características dos fármacos mais utilizados no controlo da dor (paracetamol, opióides e AINEs), contrapondo-as com as características individuais dos doentes, procurando uma medicina de precisão, tendo sido publicados 2 artigos:

- **Ribeiro, H.**, Rocha-Neves, J., Lopes-Mota, C., Teixeira-Veríssimo, M., Dourado, M., & Andrade, J. (2021). **Opióides em ambulatório na dor não oncológica: uma revisão sobre os desafios da farmacologia no envelhecimento**. *Revista Portuguesa De Medicina Geral E Familiar*, *37*(3), 233–41.

https://doi.org/10.32385/rpmgf.v37i3.12852.

- **Ribeiro H**, Rodrigues I, Napoleão L, Lira L, Marques D, Veríssimo M, Andrade JP, Dourado M. **Non-steroidal anti-inflammatory drugs (NSAIDs), pain and aging: Adjusting prescription to patient features**. *Biomed Pharmacother*. 2022 Jun;150:112958.

https://doi.org/10.1016/j.biopha.2022.112958. Q1; FI 7,419

Procurou-se depois avaliar novos alvos terapêuticos, através do conhecimento de biomarcadores incluídos nas vias de sinalização relacionadas com a apoptose, tendo sido publicado 1 artigo de revisão numa edição especial dedicada aos 50 anos da descoberta da apoptose:

- **Ribeiro H**, Sarmento-Ribeiro AB, Andrade JP, Dourado M. **Apoptosis and (in) Pain—Potential Clinical Implications**. *Biomedicines*. 2022; 10(6):1255.

https://doi.org/10.3390/biomedicines10061255. Q1; FI 4,65

Prosseguiu-se ainda com a avaliação de efeitos secundários dos opióides na última semana de vida, a necessidade de ajustes terapêuticos adequados ao máximo bem-estar do doente e dos cuidadores, tendo sido publicado um artigo original:

- **Ribeiro, Hugo**; Magalhães, Júlia; Cardoso, Tatiana; Chaves-Castro, Isabel; Lopes-Mota, Carla; Costa, Elisabete; Rocha, Patrícia; Lopes, Luísa; Bouça, Ângela; Pereira, Cristina; Paulo Andrade, José; Dourado, Marília. **Opioids and constipation therapy in the last week of life: Their impact on patients, caregivers, and the location of death**. Medicine 102(3):p e32718, January 20, 2023.

https://doi.org/10.1097/md.0000000000032718. Q3; FI 1,817

Por fim, com base nos estudos de investigação laboratorial, recolheram-se e analisaram-se os resultados dos biomarcadores no sangue periférico relacionados com a caracterização da dor, em plaquetas e monócitos:

- **Ribeiro H**, Alves R, Jorge J, Gonçalves AC, Sarmento-Ribeiro AB, Teixeira-Veríssimo M, Dourado M, Andrade JP. **Platelet Membrane Proteins as Pain Biomarkers in Patients with Severe Dementia**. *Biomedicines*. 2023; 11(2):380.

https://doi.org/10.3390/biomedicines11020380. Q1; FI 4,65

- **Ribeiro H**, Alves R, Jorge J, Gonçalves AC, Sarmento-Ribeiro AB, Teixeira-Veríssimo M, Andrade JP, Dourado M. **Monocytes in the characterization of pain in palliative patients with severe dementia – A pilot study**. *International Journal of Molecular Sciences*. 2023; 24(13):10723

https://doi.org/10.3390/ijms241310723. Q1; 6,208

Opióides em ambulatório na dor não oncológica: uma revisão sobre os desafios da farmacologia no envelhecimento

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Opioides em ambulatório na dor não oncológica: uma revisão sobre os desafios da farmacologia no envelhecimento

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RESUMO

Objetivos: Sistematizar e contribuir para o melhor conhecimento das características farmacocinéticas e farmacodinâmicas dos medicamentos opioides disponíveis em Portugal para o tratamento da dor não oncológica e, desta forma, contribuir para a escolha mais informada e adaptada ao doente, o que permitirá potenciar a eficiência, reduzindo a incidência de efeitos adversos.
Métodos: Realização de uma revisão narrativa da bibliografia sobre esta temática, utilizando os termos Medical Subject Headings (MeSH) *Analgesics, Opioid* e *Pharmacology, Clinical*. Foram também utilizados os resumos das características do medicamento de todos os opioides disponíveis em Portugal, assim como as características farmacocinéticas e farmacodinâmicas destes medicamentos registadas no *DrugBank*.
Resultados: Selecionados 22 artigos para leitura, dos quais 14 são revisões da literatura, quatro manuais de referência e quatro normas de orientação clínica, para além da utilização das bases de dados do INFARMED e do *Drugbank*. No envelhecimento há uma diminuição de cerca de 50% da taxa de filtração glomerular dos 40 até aos 70 anos, obrigando a reduções de dose e cuidados especiais na utilização de fármacos eliminados por esta via, sendo que no caso dos opioides os únicos que não têm eliminação renal são a hidromorfona e a buprenorfina. São igualmente mais frequentes a obesidade central e o aumento da α1-glicoproteína ácida, aumentando o volume de distribuição de fármacos lipossolúveis e de bases, como são os opioides, sendo que os únicos que não são lipossolúveis são a morfina e a hidromorfona. Relativamente ao metabolismo, para além de ocorrer uma diminuição do metabolismo de fase 1, a polifarmácia nos idosos é mais frequente, pelo que poderão ser preferencialmente escolhidos os opioides com metabolismo de fase 2, como o tapentadol, a hidromorfona e a morfina.
Conclusões: O reconhecimento das características individuais de cada doente idoso, associado ao conhecimento da farmacologia básica de cada opioide disponível em Portugal, poderá garantir maior segurança e salvaguardar uma maior adesão à terapêutica.

Palavras-chave: Opioides; Geriatria; Dor crónica; Farmacologia.

INTRODUÇÃO

Segundo a Organização Mundial da Saúde (OMS), o envelhecimento é "um processo de diminuição orgânica, funcional e social, não consequente de doença ou de acidente, e que ocorre inevitavelmente com o passar do tempo".[1] É sabido que, com o envelhecimento, os indivíduos apresentam particularidades e necessidades de saúde que são diferentes das apresentadas pelos mais jovens. Sendo assim, os idosos, pessoas com idade igual ou superior a 65 anos, além de cada vez mais numerosos, necessitam de abordagens médicas e terapêuticas diferenciadas que podem ser obtidas com os cuidados geriátricos.[1] Como grupo altamente heterogéneo, consideramos que não é possível balizar a decisão da escolha de um fármaco e da sua dose numa idade fixa, mas nas características individuais dos doentes.

De modo sucinto, a geriatria é o ramo da medicina que se ocupa da saúde nos aspetos clínicos, sociais, preventivos e curativos das doenças no envelhecimento.[2] Estas doenças são maioritariamente incapacitantes, crónicas e degenerativas, quase sempre acompanhadas por dor ou que contribuem para a génese da dor.[3] De facto, cerca de 50% dos idosos na comunidade e 83% se

institucionalizados têm dor crónica moderada a severa.[3] Por esta razão, o conhecimento da medicina da dor, abrangendo desde a neurofisiologia à farmacologia e terapêutica, é fundamental para uma prestação de cuidados geriátricos otimizados.

A dor crónica, como qualquer outra doença, necessita de tratamento adequado. A OMS preconiza a utilização de medicamentos opioides para o tratamento da dor moderada a severa.[4] Sabe-se que a utilização destes fármacos acarreta riscos, mas também se reconhece que os opioides disponíveis em Portugal não são iguais, apresentando características farmacocinéticas diversas.

Assim, o objetivo deste trabalho é sistematizar e contribuir para o melhor conhecimento das características farmacocinéticas e farmacodinâmicas dos medicamentos opioides disponíveis em Portugal para o tratamento da dor não oncológica e, desta forma, contribuir para a escolha mais informada e adaptada ao doente, o que permitirá reduzir a incidência de efeitos adversos e aumentar a eficiência dos cuidados. Não existia, até ao momento, uma abordagem totalmente centrada no doente como a que é proposta neste trabalho.

MÉTODOS

Para este artigo de revisão narrativa foi realizada uma pesquisa bibliográfica nas bases de dados PubMed/MEDLINE, *Database of Abstracts of Reviews of Effects, The Cochrane Library, National Guideline Clearinghouse, National Health Service Evidence* e Index das Revistas Médicas Portuguesas. Para a seleção dos artigos e outros documentos foram utilizadas equações com os seguintes termos *Medical Subject Headings* (MeSH): *Analgesics, Opioid* e *Pharmacology, Clinical*. Foram também utilizados os resumos das características do medicamento de todos os opioides disponíveis em Portugal, através de pesquisa na página eletrónica da Autoridade Nacional do Medicamento e dos Produtos de Saúde (INFARMED), assim como as características farmacocinéticas e farmacodinâmicas destes medicamentos registadas no *DrugBank*.

Critérios de inclusão

- Artigos e outros documentos publicados no período de tempo compreendido entre 1 de janeiro de 2000 a 1 de março de 2020, nas línguas inglesa, portuguesa e espanhola.
- Artigos originais, meta-análises, revisões sistemáticas e normas de orientação clínica, cujo conteúdo correspondesse aos objetivos da pesquisa.

Critérios de exclusão

- Excluídas revisões de literatura não sistematizadas e publicações que não abordassem as características farmacocinéticas e farmacodinâmicas dos medicamentos opioides ou que abordassem o tratamento de dor oncológica.
- Artigos e documentos noutras línguas, que não portuguesa, inglesa ou espanhola.

Avaliação da qualidade

Para avaliar a qualidade e interesse dos artigos fez-se inicialmente a leitura do título e resumo do artigo, excluindo os que não se relacionavam com o tema. Posteriormente foi feita a leitura dos artigos restantes na íntegra, verificando quais cumpriam os critérios de inclusão estabelecidos e quais contribuíam com informação relevante para a realização desta revisão.

RESULTADOS

Foram encontrados 42 artigos, 27 dos quais através da pesquisa pelos termos MeSH definidos e 15 artigos através de pesquisa direta nos endereços eletrónicos de sociedades relevantes e utilização de manuais de referência.

Relativamente aos 27 artigos encontrados através da pesquisa pelos termos MeSH, foram excluídos 20

1. Médico especialista em Medicina Geral e Familiar. USF Barão do Corvo.
2. Médico da Equipa Comunitária de Suporte em Cuidados Paliativos de Gaia.
3. Serviço de Angiologia e Cirurgia Vascular, Centro Hospitalar e Universitário de S. João.
4. Unidade de Anatomia, Departamento de Biomedicina, Faculdade de Medicina da Universidade do Porto. Porto, Portugal.
5. Grupo de Estudos de Cuidados Paliativos, Associação Portuguesa de Medicina Geral e Familiar. Lisboa, Portugal.
6. Serviço de Medicina Interna, Centro Hospitalar e Universitário de Coimbra. Coimbra, Portugal.
7. Unidade Curricular de Geriatria, Faculdade de Medicina da Universidade de Coimbra. Coimbra, Portugal.
8. Institute for Clinical and Biomedical Research (iCBR), Faculdade de Medicina da Universidade de Coimbra. Coimbra, Portugal.
9. Centro de Estudos e Desenvolvimento de Cuidados Continuados e Paliativos, Faculdade de Medicina da Universidade de Coimbra. Coimbra, Portugal.
10. Unidade Curricular de Cuidados Paliativos e Terapêutica da Dor, Faculdade de Medicina da Universidade de Coimbra. Coimbra, Portugal.
11. Center for Health Technology and Services Research, Faculdade de Medicina da Universidade do Porto. Porto, Portugal.

TABELA 1. Alterações farmacocinéticas mais frequentes no envelhecimento

Variável	Adultos jovens (20-30 anos)	Adultos idosos (60-80 anos)
Água corporal (% do peso corporal)	61	53
Massa magra (% do peso corporal)	19	12
Tecido adiposo (% do peso corporal)	26-33 (mulheres) 18-20 (homens)	38-45 (mulheres) 36-38 (homens)
Albumina sérica (g)	4,7	3,8
Função renal (TFG) (%) (↓0,75ml/min/ano a partir dos 35-40 anos)	100	50-60
Função hepática (atividade do CYP450/fase 1 de biotransformação)	100	50-70

Nota: Adaptado de Katzung (2014).[12]

artigos, por serem repetidos (10 artigos) e por se afastarem dos objetivos desta revisão (10 artigos), nomeadamente por se centrarem na eficácia dos opioides no tratamento da dor e nos seus mecanismos de ação (quatro artigos), em dados laboratoriais relacionados com biomarcadores (dois artigos), na dependência dos opioides encontrada nos Estados Unidos da América (dois artigos) e na dor oncológica (dois artigos). Dos 10 artigos excluídos por se afastarem dos objetivos da revisão, dois foram excluídos pela leitura do título, cinco pela leitura do resumo e três pela leitura integral, o que resultou numa seleção de sete artigos.[5-11] O mais importante e completo foi o de Petrovic (2012),[5] que abordou as reações adversas a fármacos, com um enfoque especial na prevenção.

Quanto aos restantes 15 artigos, destacam-se quatro manuais de referência na área,[2,12-14] quatro normas de orientação clínica[3-4,15-16] e quatro documentos de trabalho de sociedades científicas.[1,17-19] Dos restantes três artigos, dois foram obtidos através da bibliografia dos artigos selecionados anteriormente[20-21] e um através do Repositório do Estudo Geral da Universidade de Coimbra.[22]

Segue-se uma compilação dos elementos mais importantes coligidos durante esta pesquisa.

Particularidades da farmacologia no envelhecimento

O envelhecimento origina alterações corporais importantes para a farmacocinética e farmacodinamia.[2,5,12,20,22] Na Tabela 1 estão representadas as alterações mais frequentes, relacionadas com a absorção, distribuição, metabolismo e excreção de grande parte dos fármacos.

A absorção não é consideravelmente afetada pelo envelhecimento *per se*, mas pelo acumular de fatores que interagem com o avançar da idade, nomeadamente: alteração dos hábitos nutricionais, consumo de medicamentos de venda livre (e.g., antiácidos; laxantes), redução da motilidade gástrica e intestinal, redução do esvaziamento gástrico e aumento do seu pH, redução do fluxo sanguíneo esplâncnico, menor secreção de enzimas e atrofia da mucosa.[2,12]

Quanto à distribuição, como os idosos apresentam maior probabilidade de diminuição da água corporal, relativamente à população adulta jovem, é necessária cautela na utilização de fármacos hidrossolúveis, visto que atingirão facilmente a concentração crítica de toxicidade.[2,5,12] Por outras palavras, as doses máximas toleradas serão inferiores. Em contraste, os fármacos lipossolúveis apresentam um prolongamento do tempo de ação, relacionado com o aumento do tecido adiposo (sobretudo a nível abdominal), pelo que deve haver precaução na titulação destes fármacos e na posologia aconselhada, devendo alargar-se os períodos entre administrações.[2,5,12] Há ainda que considerar a diminuição da albumina sérica, característica do envelhecimento, e o aumento da α1-glicoproteína ácida,[2] que se repercutem na farmacocinética quer de fármacos catiónicos/ácidos [e.g., os anti-inflamatórios não esteroides (AINE)], com maior ligação à albumina, que terão tendência para reduzirem o seu volume de distribuição, quer de fármacos aniónicos/básicos (e.g., os opioides), que terão tendência para aumentar o seu volume de distribuição.[2,5,12]

Relativamente ao metabolismo, o envelhecimento causa não só a diminuição do fluxo sanguíneo hepático,[5,12] como também a diminuição da atividade de enzimas envolvidas na oxidação, redução e hidrólise (fase 1 de biotransformação, que inclui o citocromo P450), mantendo-se praticamente inalterada a fase 2 de

TABELA 2. Características farmacocinéticas dos opioides disponíveis em Portugal

Farmacocinética	Tramadol Rμ; SNRI	Codeína Rμ	Morfina Rμ; Rδ; Rκ	Fentanilo Rμ	Buprenorfina Rμ (parcial); Rκ
Absorção	I.delgado	I.delgado	I.delgado	I.delgado Pele	I.delgado Pele
Distribuição	80% Livre 20% GPA Lipossolúvel	90% Livre 10% GPA Lipossolúvel	70% Livre 30% GPA Hidrossolúvel	50% Livre 50% GPA Lipossolúvel	Livre Lipossolúvel
Metabolismo	Fase 1 M1	Fase 1 e 2	Fase 2 (90%) M3G*; M6G**; normorfina***	Fase 1 Norfentanilo (inativo)	Fase 1 3 metabolitos (inativos)
Eliminação	Renal 100%	Renal 90%	Renal 90% Biliar 10%	Renal 75% Biliar 15% (norfentanilo)	Biliar 90%

Legenda: Rμ = recetor opioide um; SNRI = inibidor da recaptação da serotonina e noradrenalina; Rδ = recetor opioide delta; Rκ = recetor opioide kappa; I. delgado = Intestino delgado; OROS = tecnologia de libertação prolongada *osmotic-controlled released oral*; GPA = α1-glicoproteína ácida; M3G = morfina-3-glucoronido; M6G = morfina-6-glucoronido; H3G = hidromorfona-3-glucoronido.
*metabolito neuroexcitatório (epileptogénico). **metabolito responsável pelo efeito analgésico. ***metabolito neurotóxico.

biotransformação (glucoronidação, acetilação e sulfatação).[2,5] Assim, os medicamentos que apresentem metabolismo hepático de fase 1 terão um prolongamento da sua ação e maior potencial tóxico, para além de apresentarem maior potencial de interações medicamentosas e alimentares.

A eliminação renal é a característica farmacocinética mais frequentemente alterada com a idade.[1,2] Depois dos 40 anos, a taxa de filtração glomerular diminui cerca de 0,75ml/min/ano,[2] ou seja, pelos 70 anos há uma redução esperada da função renal de cerca de 50%.

Relativamente à farmacodinamia, cerca de 20 a 40% dos idosos terão anualmente reações adversas a fármacos.[2,6] Os principais fatores que contribuem para esta elevada incidência de efeitos laterais são: medicação múltipla, incluindo a automedicação, maior número de comorbilidades, uma vez que o estado de doença altera a disponibilidade e/ou o efeito de um fármaco, maior afeção sistémica por estas, maior sensibilidade aos efeitos dos fármacos e farmacocinética alterada.[2,6,12]

Os idosos apresentam frequentemente reações exageradas a fármacos com efeito no sistema nervoso central (SNC), o que se deve em parte à perda de função e também à coexistência de múltiplos medicamentos nestas idades com ação no SNC, como as benzodiazepinas, os antidepressivos e os opioides.[6]

Os critérios de Beers e de START-STOPP são ferramentas disponíveis para a identificação de medicamentos potencialmente inadequados a utilizar nos idosos, particularmente na presença de determinada condição ou patologia ou ainda outra medicação.

Para aumentar a adesão à terapêutica e evitar efeitos adversos dos fármacos é fundamental respeitar algumas regras de prescrição, nomeadamente:[22]

1. Começar com doses reduzidas e titular até à dose mínima eficaz;
2. Esperar três semividas (ajustadas para a idade) antes de aumentar a dose;
3. Se a resposta não surgir, medir os níveis plasmáticos ou substituir por outro.

A escolha por um grupo fármaco-terapêutico e, dentro deste, de um medicamento particular deverá ser efetuada respondendo a alguns pontos, de que se destaca a fundamentação da eficácia, a informação sobre a segurança, a conveniência do medicamento e o custo da terapêutica.[22]

Hidromorfona Rμ (+); Rκ	Oxicodona Rμ (+); Rκ	Tapentadol Rμ; IRN
Cólon (OROS)	I.delgado	I.delgado
Livre Hidrossolúvel	60% Livre 40% GPA Lipossolúvel	80% Livre 20% GPA Lipossolúvel
Fase 1 e 2 (80%) H3G*	Fase 1 Noroxicodona; oximorfona	Fase 2 Glucoronido (inativo)
Cólon 80% Renal 20%	Renal 70% Fecal 30%	Renal 100%

Particularidades do uso de opioides no envelhecimento

Os opioides são um grupo de analgésicos com vários efeitos periféricos, espinhais e centrais, devido à ativação dos seus recetores mu (μ), kappa (κ) e delta (θ), que se encontram em todo o SNC, especialmente no núcleo do trato solitário, área cinzenta periaquedutal, córtex cerebral, tálamo e substância gelatinosa da medula espinhal. Inibem a sensibilização periférica e a condução nervosa, as sinapses nociceptivas na substância cinzenta medular e ativam a modulação descendente dependente de opioides (mecanismo mediado por serotonina, β-endorfina e dinorfina/met-encefalina).[13-14,22]

De acordo com as características dos opioides, é possível perceber que existem determinados grupos de doentes com características perante as quais devemos optar preferencialmente por determinado medicamento.[22]

Na Tabela 2 são apresentadas as características farmacocinéticas dos opioides disponíveis para uso clínico em Portugal,[23-24] as quais devem ser consideradas quando se pretendem usar estes analgésicos para tratar a dor moderada a severa em idosos.

Assim, na dor moderada para a qual a OMS aconselha a utilização de opioides menos potentes,[4] tramadol e codeína, realça-se o facto destes fármacos serem lipossolúveis, ambos em distribuição maioritariamente livre. Este facto implica o prolongamento do seu tempo de ação, o que poderá ter grande impacto nos doentes idosos obesos, sobretudo os que tenham obesidade central. O facto destes fármacos apresentarem metabolismo de fase 1 implica o agravamento do prolongamento de ação, sobretudo na população idosa, o que pode justificar e ser a razão para grande parte dos efeitos adversos reportados com estes medicamentos, neste grupo etário.[22] Ou seja, nos doentes idosos e nos doentes obesos poder-se-á considerar o alargamento dos tempos de administração de 8/8h para 12/12h. Para além disso, as doses máximas diárias não devem ser as mesmas que para a população geral. Sugerimos que devam ser diminuídas para 200mg de tramadol e 210mg para a codeína, comparativamente a 400mg e 330mg, respetivamente, nas populações mais jovens. O facto de terem eliminação renal reforça a necessidade de limitação da dose máxima, especialmente em idosos e em doentes com doença renal crónica.

Relativamente aos doentes com necessidade de opioides fortes, por dor severa, e assumindo que se abordará apenas a dor não oncológica, existem quatro medicamentos disponíveis em Portugal pela via oral: morfina, hidromorfona, oxicodona e tapentadol.

A morfina, apesar do seu baixo custo e absorção quase completa por via oral, apresenta uma biodisponibilidade de cerca de 30% pela via oral e metabolitos ativos com maior toxicidade, pelo que é uma hipótese inferior em relação aos restantes, sobretudo para os doentes idosos.[6,15,23-27]

Em relação à absorção, o único que poderá sofrer menos com as variações individuais é a hidromorfona, pois apresenta absorção cólica.[24] Como tal não é tão afetada por fatores já descritos como sendo mais comuns com o avançar da idade, como hábitos nutricionais diferentes, outros medicamentos que esteja a tomar que compitam com os mesmos locais de absorção, aumento do pH gástrico, redução do esvaziamento gástrico, redução do fluxo sanguíneo esplâncnico e menor secreção de enzimas.[7,12]

A morfina e a hidromorfona são os únicos opioides hidrossolúveis,[23-27] o que quer dizer que apresentam um

limiar de toxicidade inferior em doentes idosos, devido à menor proporção de água corporal. Em doentes obesos dever-se-á considerar que a oxicodona e o tapentadol poderão ter um prolongamento da sua ação, pelo que a posologia deve ser mais alargada, para além de que as titulações deveriam ser mais lentas.[15,22]

Relativamente ao metabolismo, o tapentadol apresenta um metabolismo de fase 2,[7,24] evitando vias enzimáticas onde são metabolizados outros fármacos, reduzindo por isso as interações. A hidromorfona também apresenta metabolismo maioritariamente de fase 2,[8,24] pelo que seria outra boa alternativa de primeira linha em doentes polimedicados.

Quanto à eliminação, o único que não apresenta eliminação renal é a hidromorfona,[23-27] pelo que deveria ser considerado prioritário em idosos e em doentes com doença renal crónica.

Segundo a OMS,[16] as regras básicas no tratamento da dor crónica implicam uma predileção pela via oral, a intervalos fixos e regulares, respeitando a escada analgésica, adaptada ao indivíduo e às suas características únicas, sempre que possível com utilização de terapêuticas adjuvantes e tendo atenção aos detalhes de cada doente e de todas as suas dimensões. Assim, apenas em alguns doentes com dor crónica tratada em ambulatório se deverá optar pela via transdérmica, para a qual existem em Portugal o fentanilo e a buprenorfina. Relativamente à farmacocinética destes dois medicamentos realça-se a sua eliminação. Enquanto a buprenorfina apresenta uma eliminação predominantemente biliar,[23-27] o fentanilo tem maioritariamente eliminação renal. No entanto, salienta-se o facto de esta eliminação renal acontecer com metabolitos inativos,[24] pelo que a função renal destes doentes está mais salvaguardada.[15] Assim, tendo em atenção a segurança farmacocinética, os dois apresentam perfis idênticos, embora alguns autores mantenham como prioritária a utilização de buprenorfina em doentes idosos e com doença renal crónica.[22]

Relativamente à farmacodinamia dos opioides, é desaconselhada a utilização concomitante de antidepressivos tricíclicos com os opioides, a utilização de opioides em doentes com quedas frequentes e/ou com demência. Estes medicamentos também só deverão ser prescritos por mais de duas semanas se acompanhados de laxante para prevenção da obstipação[17-19,22] e por medidas não farmacológicas como aumento de fibra e líquidos, que devem acompanhar a terapêutica desde o início. A prescrição crónica de opioides para a dor não oncológica é discutível, pois não apresentam eficácia demonstrada a longo prazo. Também a quantidade e a gravidade dos efeitos adversos tendem a aumentar com o tempo de utilização, motivo pelo qual deverá equacionar-se a descontinuação em cada reavaliação, que deverá ser efetuada em frequência nunca superior a três meses.[22]

Um dos conselhos para reduzir os efeitos adversos dos fármacos nos idosos é começar com doses reduzidas e titular a resposta desejada.[5] Relativamente aos opioides na população adulta preconiza-se que a dose inicial seja equivalente a 5mg de morfina oral.[13-14] No caso dos idosos é aconselhável que esta dose inicial seja equivalente a 2,5mg de morfina oral.[13,22] Esta recomendação está relacionada com o potencial iatrogénico da morfina, a que se associa o risco de iniciar uma terapêutica com opioides de libertação prolongada ou retardada.[13-14,22]

Outra das recomendações para reduzir efeitos adversos nos idosos é esperar três semividas (ajustadas para a idade) antes de aumentar a dose.[5] As características do doente são fundamentais para determinar se a semivida de um fármaco aumenta ou diminui em relação ao esperado para o adulto jovem saudável, como foi amplamente abordado neste trabalho relativamente às alterações da farmacocinética.

Por fim, outra das principais recomendações para reduzir o potencial iatrogénico dos fármacos em idosos: se a resposta não surgir, medir os níveis plasmáticos ou substituir por outro.[5] Relativamente aos opioides existem algumas razões que devem levar à rotação entre fármacos desta classe: ausência de resposta terapêutica, desenvolvimento de efeitos adversos, dificuldade com a administração da medicação (alteração do estado do doente) e outras (disponibilidade de opioides, considerações de formulário, mitos/crenças do doente e da família sobre determinado opioide).[13-14,22] Em primeiro lugar, deverá ser calculada a dose equianalgésica através de tabela de equianalgesia entre opioides.[13-14,22] No idoso, sobretudo se apresentar síndroma de fragilidade e/ou estiver acamado/muito dependente para atividades de vida diárias, deverá proceder-se a uma redução de 50% na dose.[22] Em seguida,

deverá ser efetuada uma reavaliação (esperar cerca de três semividas) psicossocial da intensidade da dor e das comorbilidades que podem implicar reajustes de cerca de 15% de 72/72 horas.[22]

DISCUSSÃO E CONCLUSÕES

A farmacologia básica é o melhor suporte de decisão clínica na utilização de fármacos, conferindo segurança na opção estratégica tomada pelo médico. É necessário conhecer as características do doente, nomeadamente dados antropomórficos (biótipo, peso, massa gorda/massa magra), função hepática e função renal, para além de antecedentes pessoais, medicação habitual, avaliação física e nutricional, mental, cognitiva e funcional o mais completas possível.

Os resultados demonstram, além da escassez de estudos nesta área específica do tratamento da dor, que o tramadol, a codeína, o tapentadol, a oxicodona, o fentanilo e a buprenorfina, sendo medicamentos lipossolúveis e com forte afinidade à α1-glicoproteína ácida, aumentam o seu volume de distribuição nos doentes obesos e na generalidade da população idosa. Ou seja, a posologia preconizada para estes fármacos deveria ser alargada, tendo em conta o prolongamento da sua ação, que é uma forte possibilidade, e as titulações devem ser mais lentas, tendo igualmente em conta a regra de aguardar por três semividas plasmáticas antes de qualquer aumento de dose.

No caso dos opioides hidrossolúveis, como a morfina e a hidromorfona, dever-se-á considerar o seu maior potencial tóxico para a maioria dos doentes idosos e doentes desidratados, ou seja, as titulações devem ser feitas de forma mais cautelosa, aumentando a dose equivalente de morfina em menor quantidade em cada ajuste. Por apresentarem um volume de distribuição e uma semivida inferior, deve ser considerada uma posologia mais estreita.

Poder-se-á ainda aferir que o tramadol, o fentanilo, a buprenorfina e a oxicodona serão os medicamentos com maior potencial de toxicidade nos doentes idosos, sobretudo os muito idosos e os polimedicados, pelo seu metabolismo de fase 1. Assim, no caso de ausência de eficácia deverão ser consideradas doses máximas inferiores para eventual rotação de opioides (que se considera realizar em dose equivalente diária de 90mg de morfina).

Outra conclusão importante deste trabalho é que a hidromorfona e a buprenorfina, apresentando uma eliminação renal residual, devem ser consideradas os opioides mais seguros em doentes com doença renal crónica e doentes idosos, sobretudo aqueles que apresentam alterações relevantes da função renal (TFG inferior a 60ml/min). O fentanilo também apresenta um perfil de segurança semelhante pois, apesar de apresentar uma eliminação renal relevante, esta ocorre para os seus metabolitos inativos, que não são nefrotóxicos. Nos restantes opioides, as doses devem ser adaptadas de acordo com a função renal que os doentes apresentam em cada momento.

Como limitação deste estudo, realce-se que o trabalho não se centrou em estudos comparativos em seres humanos, o que poderia atestar as relações apontadas de maior ou menor segurança em algumas populações especiais. Tal deve-se ao facto de não haver suficientes estudos comparativos e/ou os que existem apresentarem marcada heterogeneidade metodológica. Outra limitação importante é não considerar a eficácia dos medicamentos opioides, incluindo nos diferentes tipos de dor.

É necessária mais investigação nesta área. Com o envelhecimento populacional ter-se-á certamente um aumento exponencial de patologias que cursam com dor crónica, pelo que são cruciais abordagens eficazes e seguras baseadas na evidência.

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CONFLITO DE INTERESSES

Os autores declaram não possuir quaisquer conflitos de interesse.

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ABSTRACT

OPIOIDS IN THE AMBULATORY SETTING IN NON-ONCOLOGICAL PAIN: A REVIEW ABOUT THE CHALLENGES OF PHARMACOLOGY IN AGING

Objectives: Systematize and contribute to a better understanding of the pharmacokinetic and pharmacodynamic characteristics of opioid drugs available in Portugal for non-oncological pain and contribute to a more informed and adapted choice for the patient, which will enhance the efficiency and reduce the incidence of adverse effects.

Methods: A narrative review of the bibliography on this theme was done using the terms Medical Subject Headings (MeSH) 'Analgesics, Opioid' and 'Pharmacology, Clinical'. The Summary of Product Characteristics of all opioids available in Portugal was also used, as well as the pharmacokinetic and pharmacodynamic characteristics of these drugs registered in the DrugBank.

Results: Twenty-two articles were selected for reading, of which 14 are literature reviews, four reference manuals, and four clinical guidelines, in addition to the use of INFARMED and DrugBank databases. In aging, there is a decrease of about 50% in the renal function from 40 to 70 years old, forcing dose reductions and special care in the use of drugs eliminated by this route. Hydromorphone and buprenorphine are the only opioids that don't have renal elimination. Central obesity and an increase in acid α1-glycoprotein are also more frequent, increasing the volume of distribution of fat-soluble drugs and bases, such as opioids. Morphine and hydromorphine are the only ones that are not fat-soluble opioids. Regarding metabolism, in addition to a decrease in phase 1 metabolism, polypharmacy in the elderly is more frequent, so opioids with phase 2 metabolism, such as tapentadol, hydromorphone, and morphine, may be preferable.

Conclusions: The recognition of the individual characteristics of each elderly patient, associated with knowledge of the basic pharmacology of each opioid available in Portugal, may ensure greater safety and safeguard greater adherence to therapy.

Keywords: Opioids; Geriatrics; Chronic pain; Pharmacology.

Non-steroidal anti-inflammatory drugs (NSAIDs), pain and aging: adjusting prescription to patient features

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Non-steroidal anti-inflammatory drugs (NSAIDs), pain and aging: Adjusting prescription to patient features

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ABSTRACT

A narrative review of papers published from January 2011 to December 2021, after a literature search in selected databases using the terms "pharmacokinetics", "ibuprofen", "diclofenac", "acemetacin", "naproxen", "etodolac" and "etoricoxib" was performed. From 828 articles identified, only eight met the inclusion criteria. Selective COX-2 inhibitors are associated with higher cardiovascular risk, while non-selective COX inhibitors are associated with higher gastrointestinal risk. NSAIDs with lower renal excretion with phase 2 metabolism are less likely to induce adverse effects and drug-drug interactions. Patients with frequent NSAID use needs, such as elderly patients and patients with cardiovascular disease or impaired renal function, will benefit from lower renal excretion (e.g. acemethacin, diclofenac, and etodolac) (level of evidence 3). Polymedicated patients, elderly patients, and patients with chronic alcohol abuse will be at a lower risk for adverse effects with NSAIDs that undergo phase 2 liver biotransformation, namely, acemethacin and diclofenac (level of evidence 3). Young patients, patients dealing with acute pain, or with active and/or chronic symptomatic gastritis, selective COX-2 inhibitors (celecoxib or etoricoxib) may be a better option (level of evidence 2). Knowing the individual characteristics of the patients, combined with knowledge on basic pharmacology, offers greater safety and better adherence to therapy.

Perspective: Although there are several NSAIDs options to treat pain, physicians usually take special care to its prescription regarding cardiovascular and gastrointestinal side effects, despite the age of the patient. In this paper, based on the best evidence, the authors present a review of the safest NSAIDs to use in the elderly.

1. Introduction

According to the World Health Organization (WHO), aging is "a process of organic, functional and social decline, not a consequence of illness or accident, and which inevitably occurs over time" (WHO, 2015). It is known that, with aging, individuals present specificities and different health requirements distinguishable from those presented by younger individuals. Thus, the elderly, meaning those aged 65 years or over, are a growing group within the population that needs different medical approaches and therapeutic interventions (WHO, 2015). Since this is a highly heterogeneous group, it is not possible to decide the choice of a drug and its dose based only on age as a guide but instead on the individual characteristics of each patient.

The physical and functional characteristics of the elderly mean that diseases in aging are mostly disabling, chronic and degenerative, and almost always accompanied by pain or contributing to the genesis of pain ("[11]; Directorate-General for Health, 2013; Veríssimo et al., 2014). In fact, about 50% of the elderly in Portugal live in the community, and 83% of institutionalized have moderate or severe chronic pain (Azevedo et al., 2013). For this reason, adequate knowledge on

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pain management, ranging from neurophysiology to pharmacology and therapy, is essential for suitable and optimized treatment of pain in geriatric patients [3].

Like any other disease, chronic pain needs treatment that is suited to its pathophysiological features and intensity, among other factors. The WHO recommends the use of non-steroidal anti-inflammatory drugs (NSAIDs) for the treatment of mild, moderate and severe pain (WHO, 2009). We know that using these drugs entails risks, but we also recognize that the NSAIDs currently available to us are quite different, with distinct pharmacokinetic characteristics (Brune & Patrignani, 2015; DrugBank, 2021; INFARMED, 2021; Ribeiro et al., 2017).

Therefore, the main objective of this work is to systematize available information that would contribute to a better understanding of the pharmacokinetic and pharmacodynamic features of NSAIDs for pain management in the elderly, thus contributing to a more informed, patient-adapted choice and, consequently, reducing the incidence of adverse effects and increase the efficiency of care. The lack of information on the subject in a totally patient-centered approach that would help in daily clinical practice justified our proposition to carry out the present narrative review.

2. Methods

A literature search was carried out in Pubmed/MEDLINE, the Database of Abstracts of Reviews of Effects, Cochrane, National Guideline Clearinghouse and National Health Service Evidence (NICE) databases. For the selection of articles and other documents, equations the term Medical Subject Headings (MeSH) "pharmacokinetics" sequentially and together with the name of some of the main NSAIDs worldwide, "ibuprofen", "diclofenac", "acemetacin", "naproxen", "etodolac" and "etoricoxib" were used. Abstracts of the drug characteristics of the NSAIDs targeted in the study were also used, through a search on the website of the Portuguese National Authority for Medicines and Health Products (INFARMED), as well as their pharmacokinetic and pharmacodynamic characteristics registered in DrugBank® and Pharmgkb®.

2.1. Inclusion criteria

Articles published in English and Portuguese, from January 2011 to December 2021.

2.2. Exclusion criteria

Publications that did not address the pharmacokinetic and pharmacodynamic characteristics of NSAIDs.

Articles and documents in languages other than Portuguese or English.

2.3. Quality assessment

To assess the quality and interest of the articles, the title and abstract of the article were initially read, and those not related to the topic were excluded. Afterward, the remaining articles were read in full, verifying which ones met the established inclusion criteria and contributing relevant information to this review.

To assign evidence levels and establish recommendation strengths, the Strength of Recommendation Taxonomy (SORT) of the American Academy of Family Physicians was used (Ebell et al., 2004).

3. Results

827 articles were identified (57 in Pubmed, 742 in Cochrane and 28 in NICE), of which 800 articles were eliminated after reading the title and abstract. Of the 28 articles selected for full reading, 20 did not meet the inclusion criteria. This process resulted in 8 articles (Bacchi, 2012a; Baker & Perazella, 2020; Bally et al., 2017; Bruno A, 2014; Li, 2020; Nissen et al., 2016; Stanos, 2016; Tai & McAlindon, 2018) that met the inclusion criteria and were included to carry out this review. Guidelines and textbooks were also included (American Geriatrics Society, 2019; Cardoso, 2014; Directorate-General for Health, 2013; Directorate-General for Health, 2013; Katzung, 2004; O'Mahoney, 2015; Ritto, 2017; Veríssimo et al., 2014; World Health Organization, 2007, 2009), which addressed issues related to both the topic and objectives of the review. Fig. 1 represents the identification process of the articles used.

Globally, the cost of NSAID prescription reached 98.026 million US dollars in 2020 and is projected to rise to 125.552 million dollars by 2028 ([8].

According to a literature review carried out by Bruno et al. (2014), the analgesic benefit and anti-inflammatory properties of NSAIDs depend on COX-2 activity (Bruno A, 2014). Furthermore, a meta-analysis performed by Li et al. (2020) revealed that oral NSAIDs do not yield significant differences in terms of analgesic potency (Li et al., 2020).

50% of patients taking NSAIDs chronically develop mucosal damage of the small intestine, and 2–4% of these individuals develop GI ulcers and bleeding [16,18].

Therefore, the main issue related to the use of NSAIDs should focus on their safety profile [2,17], particularly in the elderly (Bowie, 2007; Directorate-General for Health, 2013; Li, 2020; Petrovic, 2012; Ribeiro et al., 2017), which, in turn, is related to the pharmacokinetic, pharmacodynamic and pharmacogenomic profile of each NSAID (Bowie, 2007; Petrovic, 2012; Veríssimo et al., 2014). Considering that each NSAID can exhibit a different safety profile, according to van der Petrovic (2012), it is the patient's individual characteristics that needs to be considered, since these are key to therapeutic adaptation and adequacy (Petrovic, 2012).

3.1. Features of the elderly patient that interfere with the pharmacokinetics and pharmacodynamics of NSAIDs

Aging causes important changes in the body that interfere with drug pharmacokinetics [4,9] and pharmacodynamics (Azevedo, 2012; Katzung, 2004; Petrovic, 2012; Ribeiro et al., 2017; Veríssimo et al., 2014). Table 1 shows the most frequent changes related to absorption, distribution, metabolism, and excretion of most drugs.

Drug absorption is not significantly affected by aging per se, but essentially through the adding-up of multiple factors that interact with each other as we age, namely: changes in nutritional habits, consumption of over-the-counter drugs (e.g., antacids; laxatives; and others), reduced gastric and intestinal motility, reduced gastric emptying and increased pH, reduced splanchnic blood flow, less enzyme secretion and mucosal atrophy (Katzung, 2004; Veríssimo et al., 2014).

When analyzing drug distribution, since the elderly show a decrease in body water when compared to the young adult population, caution is warranted with the use of water-soluble drugs, as they will easily reach critical toxicity concentrations (Katzung, 2004; Petrovic, 2012; Veríssimo et al., 2014). In other words, the maximum tolerated doses will be lower in geriatric age [4]. In contrast, fat-soluble drugs have a prolonged time of action due to the body's increase in adipose tissue with aging, especially abdominal fat [4]. Care must be taken in the titration of fat-soluble drugs, in the recommended/administered doses and the timing between doses, which should be extended to assure better safety (Katzung, 2004; Petrovic, 2012; Veríssimo et al., 2014). It is also necessary to consider the decrease in serum albumin, a known feature of aging, and the increase in α1-acid glycoprotein (Veríssimo et al., 2014). These aspects affect both the pharmacokinetics of cationic/acidic drugs (e.g. NSAIDs), with greater binding to albumin and the reduction of their volume of distribution, like that of anionic/basic drugs (e.g., opioids), which tend to increase their volume of distribution (Katzung, 2004; Petrovic, 2012; Veríssimo et al., 2014).

Regarding metabolism, aging causes a decrease in hepatic blood flow (Katzung, 2004; Petrovic, 2012) and a decrease in enzyme activity

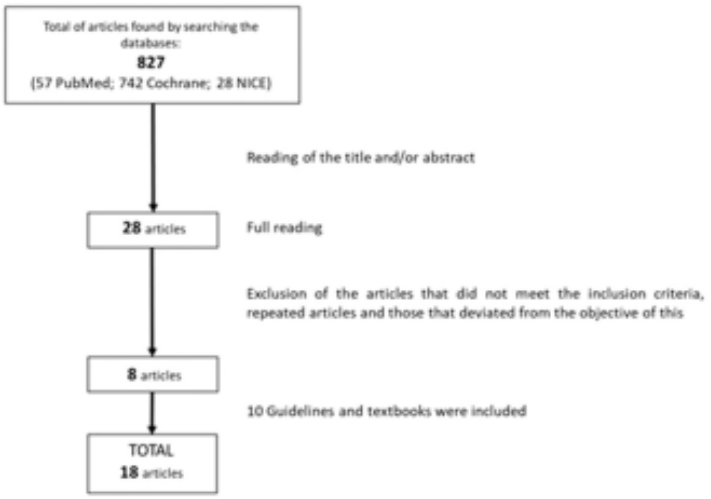

Fig. 1. Graphic representation of the identification process of articles to be included in the review through bibliographical research.

Table 1
Most frequent body changes in aging, which can interfere with pharmacokinetics, Adapted from Katzung, 2004.

Variable	Young adults (20–30 years)	Elderly (60–80 years)
Body Water (% of body weight)	61	53
Lean Mass (% of body weight)	19	12
Adipose Tissue (% of body weight)	26–33 (women) 18–20 (men)	38–45 (woman) 36–38 (men)
Serum albumin (g)	4.7	3.8
Renal function (%)	100	50–60
Liver function (CYP450 activity/phase 1 biotransformation)	100	50–70

involved in oxidation, reduction, and hydrolysis (phase 1 of biotransformation, which includes cytochrome P450 activity) [9]. The activity of enzymes involved in glucuronidation, acetylation, and sulfation (phase 2 of hepatic biotransformation) remains practically unchanged (Petrovic, 2012; Veríssimo et al., 2014). Thus, drugs that undergo phase 1 hepatic metabolism will have an extended duration of action, higher toxicity potential, in addition to presenting greater potential for drug and food interactions [9].

Renal elimination is the most frequently altered pharmacokinetic feature with age (Katzung, 2004). After age 40, the glomerular filtration rate decreases by about 0.75 ml/min/year (Veríssimo et al., 2014), so at age 70, a reduction in renal function of about 50% is expected [4].

Regarding pharmacodynamics, around 20–40% of the elderly will experience adverse drug reactions annually (Bowie, 2007; Veríssimo et al., 2014). The main factors contributing to this high side-effect incidence in the elderly are polymedication, including self-medication, and a greater number of comorbidities, with a change in drug availability/effect due to disease. This change translates into a greater systemic effect, an increased sensitivity to the drug's effect, and altered pharmacokinetics (Bowie, 2007; Katzung, 2004; Veríssimo et al., 2014).

Considering those adverse reactions, drug interactions and side effects are frequent and relevant in the elderly. Therefore, it is important to have instruments that make it possible to avoid them due to their ability to predict the onset. The Beers and START-STOPP criteria (O'Mahoney, 2015) are tools available to help identify potentially inappropriate drug use in the elderly, specifically in the presence of certain health conditions, diseases, or other drugs, thus avoiding consequences that could be disastrous for the elderly (Veríssimo et al., 2014).

To increase adherence to therapy and avoid adverse drug effects, it is essential to respect some prescription rules, namely (Ribeiro et al., 2017):

1. Start with reduced doses and titrate to the lowest effective dose;
2. Wait for three age-adjusted half-lives before increasing the dose;
3. If the therapeutic response does not appear, measure the plasma levels or switch to another drug.

Choosing a specific pharmacological group and, within it, a particular drug should be done by answering a few points in which the reasons for efficacy, the safety data, the convenience of the drug, and its cost should stand out (Ribeiro et al., 2017).

3.2. Mechanism of action and safety profile of different NSAIDs

NSAIDs inhibit cyclooxygenases-1 and – 2 enzymes (COX-1 and COX-2) [13], both responsible for arachidonic acid metabolism that results in the production of prostaglandins and thromboxane (Fig. 2) (Bacchi, 2012).

However, NSAIDs have different affinities with the COX enzyme, conditioning different potential for adverse effects (Baker & Perazella, 2020; Batlouni, 2010; Stanos, 2016).

As shown in Figs. 3 and 4, the preferential inhibition of COX-1 entails greater risks of adverse gastrointestinal (GI) effects due to the constitutive presence of this enzyme in the gastroduodenal muscle. On the other hand, the preferential inhibition of COX-2 holds greater risks of adverse cardiovascular effects (CV), essentially due to the higher concentration of this enzyme in the vascular endothelium of the kidney, which leads to the inhibition of prostaglandin production, essentially at a tubular or glomerular level, compromising the GFR and contributing to greater sodium retention, edema, thus contributing to heart and kidney failure [1,5,15]. Fig. 5 depicts the main effects of NSAIDs on the kidney (Baker & Perazella, 2020).

Table 2 identifies the main adverse effects of NSAIDs by NSAID

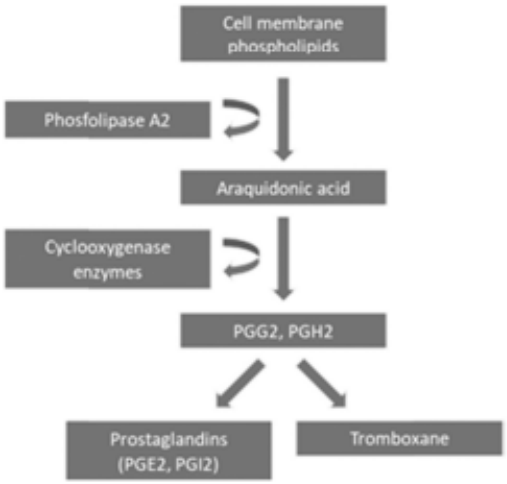

Fig. 2. Bacchi et al., 2012). Abbreviation: PGG, Prostaglandin G2; PGH2, Prostaglandin H2; PGE2, Prostaglandin E2; PGI2, Prostaglandin I2.
Breakdown of arachidonic acid into prostaglandins and thromboxane (adapted from 5.

"subclass" (Bacchi, 2012; Wongrakpanich, 2018).

The separation of NSAIDs into "subclasses" as shown in Table 2 is not consensual (INFARMED, 2021; Katzung, 2004; [10]). However, this separation helps us understand the consequences that may result from the differences in the mechanism of action of various NSAIDs, however slight.

Regarding pharmacokinetics, NSAIDs have different profiles, as shown in Table 3.

All NSAIDs directly and indirectly impact the gastrointestinal (GI) mucosa (Tai & McAlindon, 2018). It is believed that NSAID prodrugs (acemethacin) may have a less direct impact, although further studies are needed to support existing evidence (DrugBank, 2021; [6,12,14]).

Regarding distribution, fat-soluble NSAIDs, such as ibuprofen, acemethacin, and naproxen, have longer half-lives in patients with more adipose tissue, such as the obese and elderly. Regarding water-soluble NSAIDs (diclofenac, etodolac, and etoricoxib), patients with less body water may have a greater potential for toxicity (DrugBank, 2021; [4]).

Looking at metabolism, the only NSAID, among those studied here, that presents phase 2 metabolism is acemethacin (DrugBank, 2021).

Regarding elimination, the drugs with less renal excretion are, in ascending order of percentage of drug excreted by the urinary system: 1- acemethacin; 2- diclofenac; 3- etodolac; 4- etoricoxib; 5- ibuprofen; 6- naproxen (DrugBank, 2021).

A randomized controlled clinical trial involving 24081 patients demonstrated that naproxen and ibuprofen do not have a better cardiovascular safety profile than COX-2 selective NSAIDs, suggesting that non-selective NSAIDs do not have a better cardiovascular safety profile compared to COX-selective 2 NSAIDs. (Nissen et al., 2016). In this study, naproxen exhibited no cardioprotective effect, with a 25% higher mortality rate due to CV disease in the group taking naproxen. Ibuprofen had the highest rate of CV events, with the highest relative risk of major CV events. It was also observed that the risk for GI events was significantly lower with celecoxib compared to naproxen, even with a proton pump inhibitor (PPI). The risk of renal events associated with ibuprofen was significantly higher (64%); than that observed with celecoxib. Globally, naproxen and ibuprofen showed a higher risk for CV, GI, and renal

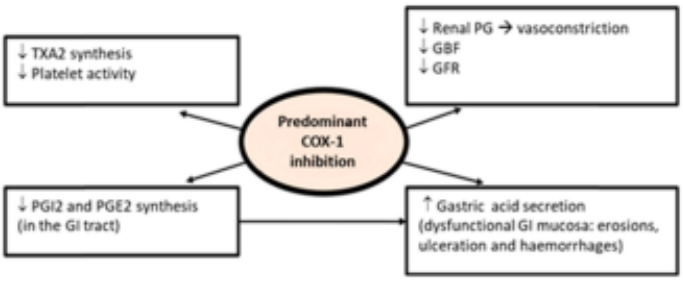

Fig. 3. Abbreviation: TXA2, Thromboxane A2; PG, Prostaglandin; PGI2, Prostaglandin I2; PGE2, Prostaglandin E2; GBF, Glomerular blood flow; GI, gastrointestinal.
Consequences of the predominant inhibition of COX-1 (adapted from Batlouni, 2010).

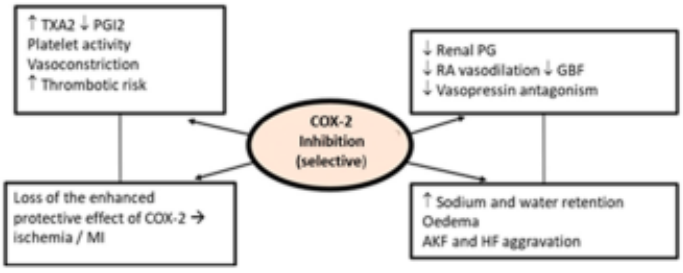

Fig. 4. Abbreviation: TXA2, Thromboxane A2; PG, Prostaglandin; PGI2, Prostaglandin I2; RA, Renal arteriole; GBF, glomerular blood flood; MI, myocardial infarction; AKF, Acute kidney failure; HF, Heart failure.
Consequences of the predominant inhibition of COX-2 (adapted from Batlouni, 2010).

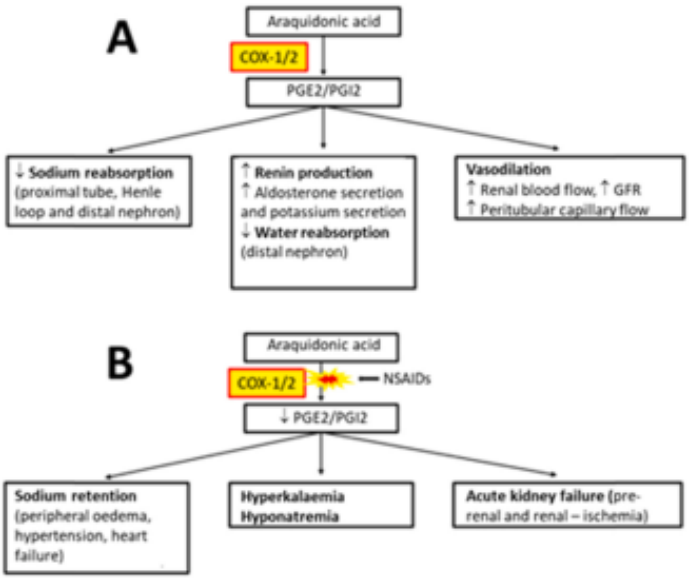

Fig. 5. 2020). Abbreviation: PGE2, Prostaglandin E2; PGI2, Prostaglandin I2; GFR, Glomerular Filtration Rate.
Prostaglandins and the kidney – A: physiological effect of PGE2 and PGI2; B: Effect of NSAIDs (adapted from Baker M, Perazella MA.

Table 2
Main adverse effects of NSAIDs by drug class, Adapted from S. Bacchi., et al., 2012 and Wongrakpanich S., et al., 2018.

Drug class	Drug	Clinical use	Adverse effects
Selective COX-1 inhibitors	Acetylsalicylic acid	antiplatelet	Gastrointestinal problems; bleeding; pulmonary, hematological and cutaneous problems (+++); cardiovascular problems (—)
Non-selective COX inhibitors	Piroxicam, indometacin, diclofenac, ibuprofen	inflammation	Gastrointestinal problems; bleeding; pulmonary, hematological and cutaneous problems (++); cardiovascular problems (–)
Selective COX-2 inhibitors	Meloxicam, etodolac, naproxen, nimesulide	inflammation	Gastrointestinal problems; bleeding; pulmonary, hematological and cutaneous problems (-); cardiovascular problems (+)
Highly selective COX-2 inhibitors	Celecoxib, etoricoxib	inflammation	Gastrointestinal problems; bleeding; pulmonary, hematological and cutaneous problems (—); cardiovascular problems (+++)

events than celecoxib.

Baly, et al. (2017) demonstrated, in a meta-analysis of individual data from Canadian and European databases, with a sample of 446,763 patients, that there was a general increase of 20–50% in acute myocardial infarction (AMI) in individuals taking NSAIDs (diclofenac, ibuprofen, naproxen, celecocib, rofecoxib). In this study, the greatest probability of increased risk was found with ibuprofen and naproxen (75%). Short-term use (8–30 days), as well as the use of higher doses of NSAIDs, showed the greatest increase in risk (doses: diclofenac > 100 mg; ibuprofen > 1200 mg; naproxen > 750 mg), (Bally et al., 2017). These results are in line with what would be expected due both to the mechanism of action and the pharmacokinetic profile of the analyzed drugs (Bruno A, 2014; DrugBank, 2021; INFARMED, 2021; Katzung, 2004).

4. Discussion

Although COX-2 selective NSAIDs are often considered to have a greater potential for CV adverse effects and some guidelines assume that naproxen is the NSAID with the best CV safety profile, current evidence demonstrates that naproxen has a worse safety profile than COX-2 selective NSAIDs (level of evidence 1) (Bally et al., 2017; DrugBank, 2021; Li, 2020; Nissen et al., 2016; [7]).

According to the pharmacokinetic profiles, we can assume that doses should be administered more sparingly in time for fat-soluble NSAIDs (ibuprofen, acemetacin, and naproxen) both elderly and obese patients,

Table 3
Pharmacokinetic characteristics of NSAIDs, (Drugbank; Pharmgkb, INFARMED 2021).

Pharmacokinetics	Diclofenac	Ibuprofen	Acemethacina	Naproxen	Etodolac	Celecoxib
Absorption	Gastroduodenal	Gastroduodenal	Gastroduodenal	Gastroduodenal	Gastroduodenal	Gastroduodenal
Distribution	Albumin (99%) Hydrosoluble	Albumin (99%) Liposoluble	Albumin (87,6%) Liposoluble	Albumin (99%) Liposoluble	Albumin (99%) Hydrosoluble	Albumin (99%) Hydrosoluble
Metabolism	Phase I e II	Phase I	Phase II	Phase I e II	Phase I e II	Phase I e II
Elimination	Renal 60% Fecal 40%	Renal 90% Fecal 10%	Renal 40% Fecal 60%	Renal 95% Fecal 5%	Renal 70% Fecal 30%	Renal 80% Fecal 20%

due to the expected extended duration of action of these NSAIDs. On the other hand, water-soluble NSAIDs (diclofenac, etodolac, and etoricoxib) should maintain the recommended rate of administration, although with a reduction in the dose suggested in the summary of the product's characteristics (SPC), due to the lower toxicity threshold seen in the elderly (strength of recommendation B) (Bacchi, 2012a; Bowie, 2007; DrugBank, 2021; Katzung, 2004; Petrovic, 2012; Ribeiro et al., 2017; Wongrakpanich, 2018; [4,9]).

Regarding metabolism, in polymedicated patients, elderly patients, and patients with chronic alcohol abuse, NSAIDs with the best safety profile will be those with phase 2 hepatic biotransformation, such as acemetacin and diclofenac (strength of recommendation C) (DrugBank, 2021; Fura, 2006; Guengerich, 2003; Wilkinson, 2005; Wongrakpanich, 2018; [3,9,11]).

As for the elimination process, the NSAIDs with the best safety profile will be those with lower renal excretion, such as acemethacin, etodolac, and diclofenac (strength of recommendation B) (Baker & Perazella, 2020; Batlouni, 2010; Bowie, 2007; DrugBank, 2021; Nissen et al., 2016; Petrovic, 2012; Wongrakpanich, 2018).

In conclusion, we can say that greater knowledge concerning a drug's pharmacological features is essential for a physician to uphold safer therapeutic choices. It also means knowing and taking into account the patient's individual features, including anthropomorphic data (biotype, weight, fat mass/mass lean); liver and kidney function; personal history; usual medication; physical, nutritional, mental, cognitive, and functional assessment, all as complete as possible.

Pharmacokinetic data should support therapeutic decision-making, namely because they help make decisions based on safety, in addition to efficacy.

In the case of NSAIDs, if no NSAID stands out for having greater efficacy, safety profiles must be carefully considered before making a therapeutic decision, always considering the type of pain eliciting treatment and individual patient features. Overall, when comparing NSAIDs, differences in pharmacokinetics and pharmacodynamics are obvious. In the absence of prospective randomized studies, drug choice can be guided by these parameters, bearing in mind risks imposed by drug-drug interactions, advanced age, organ impairment, dosage, formulation, protein binding, therapeutic half-life, and other clinically relevant pharmacological features. Current data allows NSAID prescriptions to be tailored according to specific patient needs, improving not only therapeutic outcomes but, above all, minimizing the iatrogenic risk.

More studies are needed in this area, namely controlled and randomized clinical trials that allow us to assign higher levels of evidence and strengths of recommendation.

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CRediT authorship contribution statement

Hugo Ribeiro, Conceptualization; Data curation; Formal analysis, Investigation; Methodology; Project administration, original draft; Writing – review & editing. **Inês Rodrigues:** Investigation; Methodology; Project administration. **Leonardo Napoleão:** Investigation; Methodology; Project administration. **Luís Lira:** Investigation; Methodology; Project administration. **Denise Marques:** Supervision; Validation; Visualization. **Manuel Veríssimo:** Supervision; Validation; Visualization. **José Paulo Andrade:** Writing – original draft; Writing – review & editing. **Marília Dourado:** original draft; Writing – review & editing.

Conflict of interest statement

Denise Marques is employee of Bial. Other authors have no conflicts of interest to declare.

Data availability

No data was used for the research described in the article.

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Apoptosis and (in) Pain – Potential clinical implications

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Review

Apoptosis and (in) Pain—Potential Clinical Implications

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Abstract: The deregulation of apoptosis is involved in the development of several pathologies, and recent evidence suggests that apoptosis may be involved in chronic pain, namely in neuropathic pain. Neuropathic pain is a chronic pain state caused by primary damage or dysfunction of the nervous system; however, the details of the molecular mechanisms have not yet been fully elucidated. Recently, it was found that nerve endings contain transient receptor potential (TRP) channels that sense and detect signals released by injured tissues and respond to these damage signals. TRP channels are similar to the voltage-gated potassium channels or nucleotide-gated channels that participate in calcium and magnesium homeostasis. TRP channels allowing calcium to penetrate into nerve terminals can activate apoptosis, leading to nerve terminal destruction. Further, some TRPs are activated by acid and reactive oxygen species (ROS). ROS are mainly produced in the mitochondrial respiratory chain, and an increase in ROS production and/or a decrease in the antioxidant network may induce oxidative stress (OS). Depending on the OS levels, they can promote cellular proliferation and/or cell degeneration or death. Previous studies have indicated that proinflammatory cytokines, such as tumor necrosis factor-α (TNF-α), play an important role in the peripheral mediation of neuropathic pain. This article aims to perform a review of the involvement of apoptosis in pain, particularly the role of OS and neuroinflammation, and the clinical relevance of this knowledge. The potential discovery of new biomarkers and therapeutic targets can result in the development of more effective and targeted drugs to treat chronic pain, namely neuropathic pain. Highlights: Oxidative stress and neuroinflammation can activate cell signaling pathways that can lead to nerve terminal destruction by apoptosis. These could constitute potential new pain biomarkers and targets for therapy in neuropathic pain.

Keywords: apoptosis; pain; cell signaling; oxidative stress; inflammation; biomarkers; targeted therapies; transient receptor potential (TRP) cation channels

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1. Introduction

Almost 20% of the European population suffers from chronic or intermittent pain, resulting in patient suffering and disability, and increases the economic burden on society [1]. In this context, severe pain is a major health care problem.

Neuropathic pain is a condition caused by a lesion or disease of the somatosensory system in agreement with the International Association for the Study of Pain (IASP) [2]. The neuropathic pain can be peripheral following peripheral nerve injury, postherpetic neuralgia, trigeminal neuralgia, painful radiculopathy, or painful polyneuropathy caused by metabolic and infectious diseases or chemotherapy. It can be central if associated with a spinal cord or encephalic lesion [3]. This type of chronic pain has a prevalence in the population between 7 to 10% [4,5]. Contrary to nociceptive pain, common analgesics are frequently unable to alleviate neuropathic pain. It is accompanied by hyperalgesia, allodynia, spontaneous pain, and causalgia [6], which can persist for years [7]. Therefore, neuropathic pain can become a chronic condition and hardly bearable. In extreme cases, it may lead to depression, anxiety, sleep disturbances, and impaired cognition [8].

Numerous mechanisms are involved in the pathogenesis of neuropathic pain, such as peripheral and central sensitization, changes in transient receptor potential (TRP) cation channels, ectopic activity in primary afferent fibers, and disturbance of the excitatory and inhibitory signaling [9] However, there is increasing evidence that neuroinflammation [10] and oxidative stress related to mitochondrial dysfunction and apoptosis are critically involved in inducing and maintaining neuropathic pain [9,11]. Apoptotic programmed cell death is now thought to be a principal pathway of neuronal death in many neurodegenerative diseases, including Alzheimer's, Parkinson's, and Huntington's diseases, and also in neuropathic pain [12]. Mitochondria have a central role in apoptosis [12] as the permeabilization of the mitochondrial membranes leads to the release of cytochrome c and of the complex formation that begins the cleavage and activation of proteases known as caspases [13], inactivating molecules essential for cell survival. Other molecules that mediate a program of cell suicide are activated [13]. The reduction of the membrane potential of mitochondria, the generation of free radicals and reactive oxygen species (ROS), and intracellular acidification are some other features of apoptosis [12]. Supporting the idea that apoptosis occurring in sensory neurons may contribute to neuropathic pain conditions, multiple apoptosis-related genes were observed to be upregulated in the dorsal root ganglion in a spinal nerve ligation model of neuropathic experimental pain [14].

Our aim with the present narrative review is to understand the fine molecular mechanisms involving apoptosis and neuropathic pain and the relationship to nociceptive signaling, oxidative stress, and neuroinflammation. Special attention is paid to the clinical relevance of biomarkers and the development of potential new therapeutic targets.

2. Definition and Mechanisms of Pain—A Brief Review

The IASP conducted a review about the definition of pain in 2020, and it is now considered as an "unpleasant sensory and emotional experience associated with, or resembling that associated with, actual or potential tissue damage" [15].

It was Charles Sherrington, winner of the Nobel Prize in Medicine in 1932, who divided for the first time the mechanisms of propioception, exteroception, and interoception [16], with pain, temperature, and pressure also part of exteroception [17].

Nociception refers to the processing of a noxious stimulus resulting in the perception of pain by the brain. Various neurotransmitters, nociceptors, and cellular components are involved in the mechanisms of transduction, perception, transmission, and modulation of pain [18], as shown in Figure 1.

Transduction is the conversion of a noxious stimulus (mechanical, chemical, or thermal) into electrical energy by a peripheral nociceptor (free afferent nerve ending). Transmission describes the propagation through the peripheral nervous system via first-order neurons. Modulation occurs when first-order neurons synapse with second-order neurons in the dorsal horn cells of the spinal cord, and perception is the cerebral cortical response to nociceptive signals that are projected by third-order neurons to the brain [19].

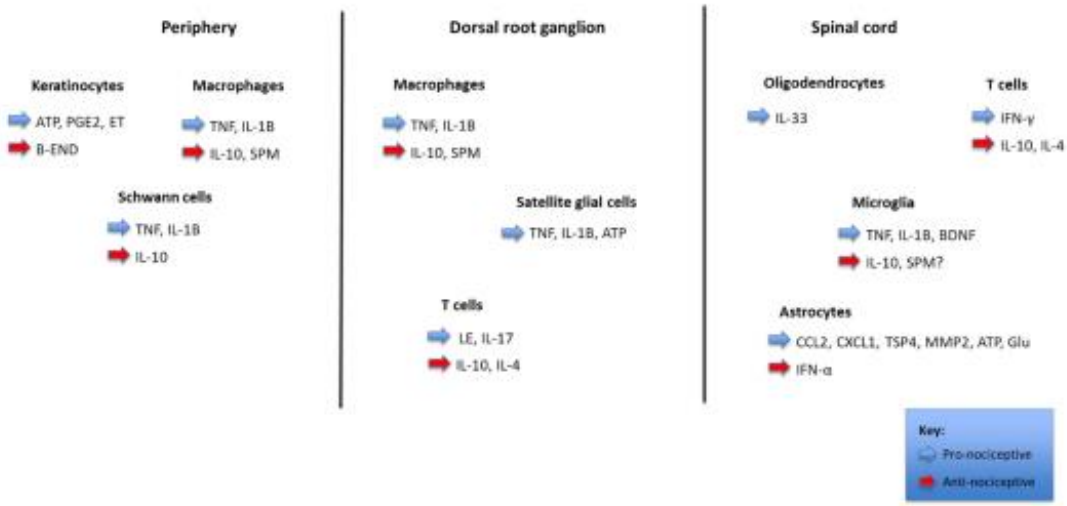

Figure 1. Pain regulation by non-neuronal cells and inflammation. The figure shows the interactions between distinct parts of a nociceptor (periphery, dorsal root ganglion, and spinal cord) with different types of non-neurons cells (keratinocytes, Schwann cells, satellite glial cells, oligodendrocytes, and astrocytes), immune cells (macrophages, T cells, and microglia), cancer cells, and bone marrow stem cells. These cells produce pronociceptive (highlighted in blue) and antinociceptive (highlighted in red) mediators, which modulate the nociceptor sensitivity and excitability through binding to their respective receptors. In the spinal cord dorsal horn, the central terminal of the nociceptor forms a nociceptive synapse with a postsynaptic neuron to mediate pain transmission in the Central Nervous System (CNS).

Pain depends on a multifactorial system with multiple pathways for numerous cortex areas, the cortical body matrix [20], which explains why it is a multidimensional experience that can significantly impair an individual's quality of life [21].

The most abundant pain sensors are in the skin and are called the transient receptor potential cation channels (TRP) [22]. The TRP family contains many members, such as the vanilloid receptors (TRPV), the canonical receptors (TRPC), the ankyrin repeat receptors (TRPA), the polycystin recaptors (TRPP), the melastatin receptors (TRPM), and the mucolipin receptors (TRPML) [22]. TRP channels are activated by histamine, bradykinin, fatty acid metabolites, endocannabinoids, cannabinoids, adenosine triphosphate (ATP), acid, heat, cold, prostaglandins, cytokines, capsaicin, monoterpenoids, among other stimuli. The initial sensation can be heat, cold, pain, or itching that can be several minutes in duration. Usually, prolonged activation of the TRP channels (for more than 20–30 min or so) results in long-term deactivation and pain relief [22]. Recently, nine TRP channels (TRPA1, TRPC5, TRPM2, TRPM4, TRPM7, TRPV1, TRPV2, TRPV3, and TRPV4) have been demonstrated to be activated by oxidative stress [23] and in neurons related to nociception (as in dorsal root ganglion (DRG) and trigeminal ganglia neurons), the expression levels of four TRP channels (TRPA1, TRPM2, TRPV1, and TRPV4) are high [11]. From [24,25], TRPV1 and TRPA1 have been demonstrated in neuropathic pain associated with diabetes or the administration of chemotherapeutics, probably mediated by the synthesis of reactive oxygen and nitrogen species [26,27], which are well-known TRPA1 activators [28,29]. Further, TRP channels have been associated with pain in neurodegenerative disorders (NDD) through oxidative stress-induced cell death, particularly related to inflammation and calcium homeostasis deregulation [29]. All these processes are connected with microglial activation, a proposed mechanism inductor of inflammation and neuropathic pain [30], which leads to neuronal

degeneration and cell death in NDD, and simultaneously to neuropathic and inflammatory pain [31,32].

Mitochondria play important roles in a myriad of cellular processes, and mitochondrial dysfunction has been implicated in multiple neurological disorders [7]. The five major mitochondrial functions (the mitochondrial energy generating system, ROS generation, mitochondrial permeability transition pore, apoptotic pathways, and intracellular calcium mobilization) may play critical roles in neuropathic and inflammatory pain [7].

Oxidative stress is a central mediator of apoptosis, neuroinflammation, metabolic disturbances, and bioenergetic failure in neurons [33]. Oxidative stress and apoptosis, as observed in the DRG neurons, play an essential role in inducing and developing pain hypersensitivity [34].

It should also be noted that apoptosis is also regulated by proinflammatory cytokines, such as Interferon gamma (IFN-g), tumor necrosis factor-α (TNF-α), interleukina-1 beta (IL-1 b), and IL-6 [35]. The roles of IL-1 and TNF- α in apoptosis in neurodegenerative diseases such as Alzheimer's disease were previously described [35,36]. More importantly, it is known that these proinflammatory cytokines have crucial roles in the modulation of neuropathic pain [35,37].

3. Apoptosis Pathways as Mediators of Pain Formation

3.1. Apoptosis Cell Signaling Pathways

The homeostasis of a multicellular organism depends on cell turnover, as it is related to the capacity to maintain the equilibrium between cell proliferation and death [38,39].

Physiological cell death occurs primarily through an evolutionary and conserved form of cellular suicide, called apoptosis (from the Greek word for decay, as with leaf fall, for example), in response to a series of intrinsic and extrinsic stimuli [38,39]. It was discovered in 1952. However, in 1972, the Australian pathologist John FR Kerr and the Scots Andrew H. Wylie and Alistair R. Currie showed the importance of this cell death process in developing an adult organism [38]. Unlike the other type of death, cell necrosis, in which the cell is a passive victim, cell apoptosis is an active form of death, in which the cell expends energy to carry out this genetically programmed cellular suicide process. For this reason, it is often called programmed cell death [38,40].

The induction of apoptosis can be divided into three steps: the inducing agent's interaction with the cell, the biochemical transduction of the death signal, and the execution by the apoptotic machinery. Different extracellular signals, such as an increase in ROS production [41] and/or a decrease in antioxidants resulting in oxidative stress, inflammatory mediators such as TNF- α and IL1-β [42], moderate hyperactivity stimulation of the N-methyl-D-aspartate (NMDA) receptors [1], and the endoplasmic reticulum stress [43] could activate signal transduction pathways that converge on a final common pathway leading to the execution phase of cellular apoptosis. At least two pathways are involved, the mitochondria-dependent pathway (intrinsic or mitochondrial pathway), which is mediated by mitochondria and the B-cell lymphoma-2 (BCL-2) family proteins, and the extrinsic or membrane pathway involving external signaling via membrane receptors from the tumor necrosis factor (TNF) family, such as TNF-R1, FAS, TNF-apoptotic inducing ligand receptors 1 and 2 (TRAIL-R1 and 2, or death receptors DR4 and 5 [44], and the low-affinity nerve growth factor (NGF) receptor, p75-NTR [27,45]. Moreover, the p38MAPK pathway causes the activation of apoptosis in neuronal cells, inducing pain (such as bone cancer pain-related hyperalgesia) [46].

However, all the pathways culminate in the activation of a series of cysteine proteases, called caspases. The effector caspases, such as caspase 3, cleave a wide variety of protein targets that are responsible for the characteristic changes in apoptotic cells. These include cell shrinkage, nuclear condensation, membrane blebbing, fragmentation into apoptotic bodies, and membrane changes that can lead to phagocytosis of the affected cell [25,33]. An alternative, proinflammatory form of apoptosis [40] in macrophages infected with Salmonella or Shigella, termed "pyroptosis" [47]. Since its discovery, pyroptosis has been

observed in the central nervous system [48] and the cardiovascular system, suggesting that this form of cell death is biologically significant. [46,47].

The knowledge of this active physiologic mode of cell death has profound implications on our understanding of several diseases. In fact, the deregulation of apoptosis is involved in the development of several diseases, such as cancer [49], neurodegenerative diseases as Alzheimer's and Parkinson's, and autoimmune or rheumatoid arthritis. Recent evidence suggests that apoptosis may be involved in pain, namely neuropathic pain [1].

The involvement of apoptosis in pain and the clinical relevance of this knowledge in the discovery of new potential biomarkers and therapeutic targets may be useful for the development of more effective and targeted drugs to treat pain.

3.1.1. The Extrinsic or Membrane Pathway of Apoptosis

The activation of membrane receptors of the TNF family, namely TNF-R1, FAS, TRAIL-R1/R2 (or death receptor 1 and 2, DR1/2) [44] and NGF-R/p75-NTR [45] by the respective ligands, TNF-alpha, FAS-L, TRAIL, and NGF, triggers the activation of enzymes called caspases, which are the effectors of the apoptotic process (Figure 2).

These cell surface receptors are mainly type I transmembrane proteins (TRAIL-DR4 and DR5 are the exception, as they are type II), which have cysteine residues in their extracellular domain. They are activated by oligomerization upon binding of the respective ligand to their extracellular domain. The cytoplasmic domain of these TNF family receptors shares a sequence with about 60 to 70 amino acid residues, which has been called the death domain. This death domain interacts with other proteins possessing the same domain, namely with FADD (FAS-associated death domain) proteins, in the case of FAS, or TRADD (TNF receptor-associated death domain), in the case of TNF-R1. For apoptosis to occur, an N-terminal death domain effector is required, through which these proteins bind to identical amino acid sequences in other proteins, recently identified as procaspase 8 that is cleaved with the consequent release of the active caspase 8 (Figure 2). Subsequently, caspase 8 activates caspase 1, formerly called ICE (interleukin converting enzyme). Caspase 1, in turn, activates caspase 3 by cleaving the respective procaspase, which is the true executor of apoptosis (Figure 2). The activation of caspase 3 leads to the inactivation of a factor that inhibits deoxyribonucleic acid (DNA) fragmentation, leading to its cleavage by the endonuclease responsible for the internucleosomal cleavage of chromatin, characteristic of cells undergoing apoptosis.

The low-affinity p75 neurotrophin receptor (NGF-R/p75-NTR) also activates the extrinsic or membrane pathway of apoptosis through its ligand, the nerve growth factor (NGF). NGF is one of the neurotrophins (NTs) family members, discovered in 1950, and is essential for the neuronal survival, growth, and development of neurons as well as the maintenance of neuronal phenotype in the mature nervous system via interaction with specific nerve surface receptors, such as tyrosine receptor kinases A (TRK-A). Although NGF is a well-known neurotrophic factor, it also acts as a mediator of pain (namely in orofacial pain), itching, and inflammation by inducing cell death through the low-affinity p75 neurotrophin receptor (NGF-R/p75-NTR), which activates caspase 8 (Figure 2) [45].

3.1.2. The Intrinsic or Mitochondrial Apoptotic Pathway

In view of the above, caspase 8 appears to be the first direct signaling link between events at the plasma membrane level and the apoptotic execution machinery. Alternatively, caspase 8 can act on the so-called mitochondrial transient permeability pore (MTPP) through BIDt, linking mitochondria to apoptotic execution. This binding was initially suspected after the existence of a protein in the inner/outer membrane of the mitochondria with apoptotic inhibitory functions, the BCL-2 protein, was demonstrated [50,51]. Other members of this family of proteins with antiapoptotic functions include the BCL-XL, BCL-W, MCL-1, and A1 proteins, among others. Contrary to the action of these proteins, there are others with proapoptotic functions, of which we highlight the BAX, BAD, BID, BIK, and BAK proteins, also members of this same family, but for which increased expression leads

to cell death. Although the BCL-2 protein appears to be exclusively membrane-bound, particularly in the mitochondria, other related proteins, such as the BID and BAD proteins, are cytoplasmic, and are transported to the mitochondria during the process of apoptosis [52]. The change in mitochondrial membrane potential with the opening of the MTPP leads to the release of cytochrome c, a mitochondrial inner membrane protein, into the cytoplasm, which induces cell death by apoptosis. Cytochrome *c* binds to apoptotic protease activating factor (APAF-1), leading to a conformational change in the latter and forming a protein complex with activated caspase 9, called the apoptosome. Caspase 9 cleaves and activates other effector caspases, namely caspase 3, as mentioned above, triggering cellular apoptosis. In turn, caspases are inhibited by the inhibitor of apoptosis proteins (IAPs), such as survivin and XIAP (X-linked IAP), which are modulated by the proapoptotic proteins, SMAC (second mitochondria-derived activator of caspase) and DIABLO (direct inhibitor of apoptosis-binding protein with low PI) (Figure 1) [53–55]. Thus, the susceptibility of a cell to enter into apoptosis is determined, in part, by the relative concentrations of the pro and antiapoptotic proteins, namely the BCL-2 family members.

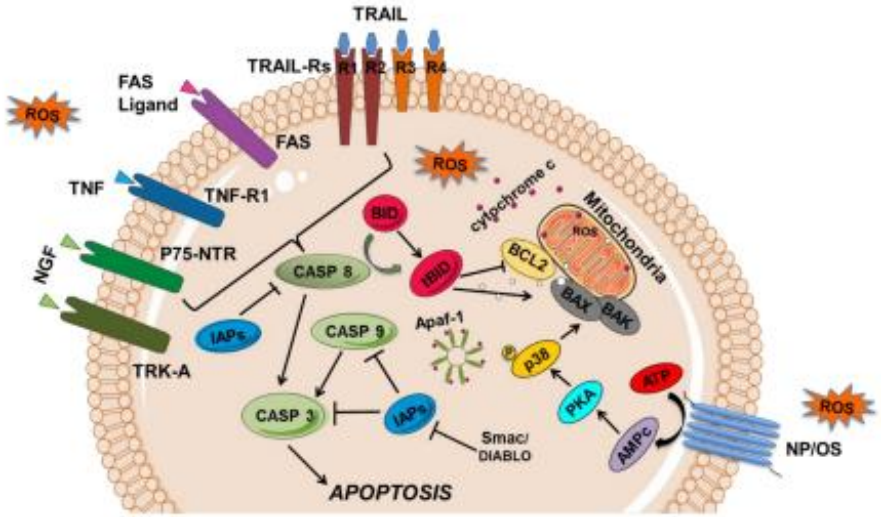

Figure 2. Representation of cell signaling pathways leading to apoptosis. Apoptosis can result from the activation of the extrinsic and intrinsic apoptosis pathways. The extrinsic or membrane pathway starts by the interaction of a death ligand from the TNF (tumor necrosis factor) family, TNF, FAS-L, TRAIL (TNF related apoptotic inducing ligand), and NGF (nerve growth factor) to the respective receptors, TNF-R1, FAS, TRAIL-R1-R2, and p75-NTR, which subsequently activate a caspase cascade (caspase 8 and 3). In mitochondria-mediated apoptosis or intrinsic pathway, proteins of BCL-2 family (BAX, BAK; BCL2) and cytochrome c are released, binding to the apoptotic protease-activating factor-1 (APAF-1) and caspase 9 (CASP 9) forms a complex called the apoptosome, which is capable of recruiting caspase-9, -3 (CASP3) and -7, and inducing apoptosis. Several apoptotic regulators are also represented, such as the CASP inhibitors (namely the IAPs—inhibitor of apoptosis proteins) and the IAPS inhibitors SMAC/DIABLO (second mitochondria-derived activator of caspase/direct inhibitor of apoptosis-binding protein with low pI). In neuropathic pain (NP), oxidative stress (OS)/ROS generated exogenously or endogenously can activate p53, which activates the proapoptotic BCL-2 proteins that can inhibit the functions of the antiapoptotic proteins, such as BAX and BAK. Besides, OS can induce nerve apoptosis by activating BAX through protein kinase A (PKA) and p38 pathway. Transmembrane death receptors such as FAS, TRAIL-R1/2, and TNF-R1 can also be activated by ROS.

Death pathways may also be triggered by excessive stimulation of the NMDA receptors that may result in necrosis (strong receptor hyperactivity) or apoptosis (moderate receptor hyperactivity), making NMDA receptors potential therapeutic targets in pain by using antagonists of these receptors to reduce excitotoxic neuronal death [1]. Moreover, p73, a pivotal gene in DNA damage belonging to the p53 family, regulates neuronal cell differentiation and oligodendrocyte precursors. This gene regulates the transcription of two protein isoforms with opposite effects on the regulation of apoptosis: TAp73, with proapoptotic function, and DeltaNp73, with antiapoptotic activity. The balance between the two protein isoforms and, thus, the control of cell proliferation and death are affected by the ubiquitin–proteasome degradation system and mediated by ligases of NEDD family [1].

As previously mentioned, oxidative stress (OS)/ROS, generated exogenously or endogenously, are involved in neuropathic pain (NP) through the intrinsic and extrinsic apoptotic pathways. Further, TRPM2 and TRPM7 channels can induced cell death mediated by oxidative stress (which could be also be related to inflammation), contributing to the observed relationship between pain, neurodegeneration, and TRPs [29,46,56].

3.2. Ubiquitin Proteasome Pathway and Apoptosis

Regulatory proteins involved in normal cell proliferation and differentiation are degraded by a proteolytic pathway, which involves lysosomal enzymes, and a non-lysosomal pathway, known as the ubiquitin–proteasome pathway (UPP) [56].

The UPP encompasses several enzymes, namely the ubiquitin-activating enzyme (E1), the ubiquitin-conjugating enzyme (E2), and a ligase (E3) that catalyzes the binding of ubiquitin to the target protein, the deubiquitinating enzyme (DUB), and proteasomes (namely the 26S proteasome) [57].

The degradation of proteins by the UPP involves two successive and distinct steps: first, several ubiquitin molecules bind to the target protein to be subsequently degraded in the proteasomes. Some of the regulatory proteins with levels are controlled by this degradative pathway include proteins involved in cell death by apoptosis, namely the BCL2 family proteins such as BAX protein [56], and the C-terminal fragment of the BID protein the BIDt [58], and the IAP family and the inhibitor of the NF-kB, IkB [59,60].

The UPP is relevant in neural development and brain structure and also in maintenance of their functions, and is implicated in synaptic plasticity, formation, and maintenance of memory. Recently, it was found that the dysfunction of this proteasomal degradation pathway leads to changes in cell death by apoptosis in diverse cell types and may be implicated in the pathogenesis of several diseases, either through hypofunction, as in neurodegenerative diseases [61,62], or by hyperfunction, as in the case of neoplastic diseases [57,62]. More recently, ubiquitin system dysfunction has been implicated in chronic pain, mainly in neuropathic pain (NP) and inflammatory pain, through ubiquitination modified protein receptors and ion channels to affect synaptic activity and efficiency [57].

One of the most important enzymes is the ubiquitin-conjugating enzyme E2B (Ube2b), also known as RAD6B. Ube2b is a member of the ubiquitin-conjugating enzyme family that has been identified to be vital in neural DNA double-strand DNA breaks (DSBs). Further, the Ube2b modulates the DNMT3a (DNA methyltransferase 3 alpha) ubiquitination degradation and *CaMKK1* (calcium/calmodulin-dependent protein kinase) gene promoter demethylation increasing CaMKK1 level. Moreover, the depletion in Ube2b reduces H2B (histones H2B) ubiquitination, contributing to elevation of the instability of the genome and neurodegeneration [62]. These studies indicate that Ube2b is essential in modulating nerve function, being a potential target to treat neuropathic pain. However, the role of Ube2b in neuropathic pain is not yet clarified [63]. Other UPP enzymes are the E3-ubiquitin protein ligases, a group of proteins that mediate ubiquitination and regulate chronic inflammatory pain via controlling the level of substrate proteins and then possibly adjust synaptic efficacy. The roles of these enzymes are being studied in inflammatory pain. The deubiquitin enzymes (DUBs) are ubiquitin-specific proteases that remove the ubiquitin groups from the degraded target proteins, improving protein stability. One of these DUBs is USP5,

which increases the Cav3.2 protein level and calcium current contributing to inflammatory pain. The knockout of UPS5 by shRNA can increase Cav3.2 ubiquitination, decrease Cav3.2 protein level, and decrease calcium current. Thus, targeting Cav3.2 deubiquitination by USP5 is a potential therapeutic target for pain associated with inflammatory disorders [62].

Moreover, the nuclear factor-κB (NF-κB) is a key regulator of molecules and pathways important for inflammation and pain [64–66]. NF-κB can exist as homo or heterodimers composed of the Rel family proteins (the p65/p50 subunits in humans) and is bound to the κB inhibitor (IκBα) in the cytoplasm, when in an inactive state. Upon activation by proinflammatory cytokines and growth factors, among others, the IκB kinase (IKK) phosphorylates IκBα, targeting its degradation in the UPP. As a result, IκBα releases the p65/p50 complex, which subsequently translocates to the nucleus and initiates the transcription of several genes, including inflammatory genes such as TNFα, IL-1β, and cyclooxogenase-2 (COX-2) that directly or indirectly influence pain [64–66]. Thus, targeting the NF-kB pathway, namely by inhibiting the IkB degradation in the UPP could be a new therapeutic approach to treating pain [43,64,65].

3.3. Endoplasmic Reticulum Stress Signalling and Apoptosis

Endoplasmic reticulum (ER) stress causes the activation of caspase signaling pathway-dependent apoptosis in neuronal cells and induces bone cancer pain-related hyperalgesia.

The ER is the cellular organelle in which protein folding, lipid biosynthesis, and calcium storage take place. Many factors can cause an imbalance in the homeostasis of ER function, resulting in ER stress. Such ER stress initiates an evolutionarily conserved signaling cascade called the unfolded protein response (UPR), which is a self-protective signaling pathway. The accumulation of unfolded proteins in the ER can activate the UPR to restore ER function. Besides the increase in ER stress as a self-protective signal transcription pathway after mild injury, the failure of this system to relieve prolonged or excessive ER stress may cause cell apoptosis, and plays an important role in neuropathic pain, specifically in rat models of bone cancer pain [43].

3.4. Oxidative Stress

When the synthesis of oxidative species is superior to the capacity of the cells to counteract them, there is the occurrence of oxidative stress. The generation of reactive oxygen species (ROS) in mitochondria is a consequence of oxidative phosphorylation related to the respiratory chain [67]. ROS acts physiologically as signaling molecules, but when produced in abundance has deleterious consequences to DNA, protein, lipids, initiation of inflammatory events, excitotoxicity, and apoptosis [6].

Mitochondrial dysfunction, the increase in ROS and reactive nitrogen species (RNS), is involved in the pathogenesis of chronic neuropathic pain [68], leading to the degeneration of primary afferents [69], and ROS accumulation originates a feed-forward mechanism of nitroxidative lesion that triggers proapoptotic factors [70,71].

In fact, mitochondrial dysfunction is observed in various neuropathic pain phenotypes, such as chemotherapy-induced neuropathy, diabetic neuropathy, HIV-associated neuropathy, Charcot–Marie–Tooth neuropathy, and trauma-induced painful mononeuropathy [71].

More recently, it was shown that damage to mitochondria and aberrant mitochondrial transport in peripheral neurons are common features of peripheral neuropathy [72]. The complex and highly energy-consuming processes of neurotransmission by peripheral neurons are critically dependent on mitochondrial metabolic functions [73]. Moreover, peripheral neurons are primarily reliant on axonal transport of mitochondria, which facilitates the rapid movement of mitochondria to areas of high-energy demand along axons up to one meter in length. A key regulator of both mitochondrial movement and function is the epigenetic modifier histone deacetylase 6 (HDAC6) [72]. The accumulated evidence shows that HDAC6 inhibition is strongly associated with alleviating peripheral neuropathy and neuropathic pain, and mitochondrial dysfunction in vivo and in vitro models of peripheral neuropathy [72]. Although it is accepted that free radicals and the altered

lipids and proteins have a crucial role in developing peripheral and central sensitization, the fine molecular mechanisms regulating their action in pain processing have not been elucidated [24] in man.

Some experimental studies demonstrated that the increase of ROS levels induces nociception [74,75]. However, removing excessive ROS or RNS by free radicals scavengers, a decrease in neuropathic and inflammatory pain is achieved [7,76,77].

Concerning the synapses at the spinal cord level, laboratory studies demonstrated that ROS increases excitatory signaling [78] using several pathways, including NMDA receptor activation [77] and the alteration of the proteins involved in glutamatergic homeostasis [79]. ROS-induced pain sensitivity that activates NMDA receptors increases Ca^{2+} influx to the cytoplasm, increasing mitochondrial ROS production and pain enhancement of neurons [7]. In contrast, there is a decrease in inhibitory transmission due to a reduction of Gamma-Aminobutyric Acid (GABA) release or death of GABAergic neurons [26].

ROS can also activate directly and indirectly the Ca^{2+}-permeable channels [23], members of the TRP family, known to be involved in neuropathic pain [24,29]. Members of the subfamilies A (TRAP1), M (TRPM2 and 7), and V (TRPV1 and 4) have been demonstrated to play a role in nociception mediated by sensory neurons [24]. These channels are expressed in primary afferent nociceptors, abundant in the skin, and related neurons [24,80]. TRP channels allow calcium to enter the nerve terminals, activating apoptotic mechanisms leading to nerve terminal degeneration [81].

Finally, oxidative stress is involved in neuroinflammation by modulating neuroinflammatory signaling involving the NFKB, MAPK [82,83], TNF-α [83], and nuclear factor erythroid 2 (NFE2)-related factor 2 (NRF2) signaling pathways [84]. NRF2 is a transcription factor encoded by the *NFE2L2* gene that remains bound to Kelch-like ECH-associated protein 1 (KEAP1) in the cytoplasm that ultimately leads to proteasomal degradation. During peripheral neuropathy, NRF2 can translocate to the nucleus and binds to antioxidant response elements (AREs), leading to the transcription of several antioxidative enzymes and cytoprotective proteins that can ameliorate neuropathy and neuropathic pain in rodent models. Thus, modulating NRF2 activity is a promising pharmacological approach in inflammatory and painful diseases [85–87].

Rodrigo Sandoval et al. (2018) demonstrated that TNF-α increased ROS production in nociceptive neurons and p35 expression followed by TRPV1 phosphorylation and an increase in Ca^{2+} influx in nociceptive neurons and increased pain sensation, which may be targeted therapeutically [83].

3.5. Inflammation

For five decades, apoptosis has been recognized as fundamental for almost all the critical biological processes that occur in multicellular organisms, since embryogenesis and throughout life, to maintain the homeostatic balance of tissues and organs as well as the progression and final resolution of inflammation [88–90].

However, this physiological form of cell death was very early on associated with the absence of an inflammatory reaction by the surrounding tissues. This was initially explained by the preservation of membrane integrity in such a way that cellular content does not come into contact with neighbouring cells [87,88,91–94].

Inside, they contain well-preserved organelles and nuclear fragments, which will be prompt digested and removed from tissues by professional phagocytes or non-professional neighboring cells that specifically recognize apoptotic cells [87,92].

Although initially it was considered that the maintenance of the integrity of the cellular membrane was the only reason why apoptosis did not cause inflammation, it is now known and consensual that the mechanisms involved in the recognition and subsequent phagocytosis and clearance of apoptotic cells will determine whether apoptosis is immunologically "silent" or anti-inflammatory [93–98].

It seems clear that during apoptosis, the inflammatory response could be blocked [98] Regarding biochemical explanation, the fact that the inflammatory response is absent dur-

ing apoptosis was initially explained by the exposure of phagocytes to phosphatidylserine after a process of translocation from the inner to the outer leaflet of the cell membrane ([99] This "eat me" signal that attracts and allows the recognition of apoptotic cells and their subsequent elimination plays a central role in phagocytosis and is one of the hallmarks of apoptosis [97,100,101].

Caspase activation, changes in mitochondrial membrane potential, and DNA cleavage are also players in apoptosis, as they contribute to disrupt cellular functions and mark cells for a "silent" phagocytic clearance [102,103].

Due to activation of caspases that cleave membrane channel proteins results in the release of adenosine monophosphate (AMP) [103].

Then, through the 5′-nucleotidase a phosphate group is removed from the AMP to produce adenosine that binds to the A2a receptors on the surface of phagocytes to generate the anti-inflammatory response [103].

Currently, other molecules, such as lysophosphatidylcholine, ATP and uridine 5′-triphosphate (UTP), sphingosine-1-phosphate, CX3CL1/fractalkine, thrombospondin-1, monocyte chemoattractant protein-1 (MCP-1), lactoferrin, are also known as effective in the "silent" clearance of apoptotic cells [104] These molecules act by sending chemotactic signals that attract phagocytes that recognize apoptotic cell-associated molecular patterns (ACAMPs) on the surface of apoptotic cells, therefore contributing to the anti-inflammatory characteristic of apoptosis. Besides, apoptotic cells can stimulate macrophages to generate anti-inflammatory mediators such as IL-10 or TGF-β, prostaglandin E2, and platelet activating factor (PAF) [105–109].

Considered as a type of physiological cell death, apoptosis also has a vital role in the responses to tissue injury or infection, determining the tissue's ability to repair and regenerate after injury [97,110–112].

It is also important in controlling the number of inflammatory cells present at sites of inflammation. The fact is that many factors and signaling pathways that are activated and intervene in the inflammatory response are also involved in the regulation of apoptosis, and vice versa [108,113,114].

Thus the concept that apoptosis is non-inflammatory is not always a correct one [115] For example, it was observed in an experimental model that the injection of agonistic antibody against the Fas receptor causes hepatocytes apoptosis and inflammation response. In the same experimental model, the authors also observed that if they inhibited or prevented apoptosis by administering a caspase-3 inhibitor, hepatic inflammation would also be reduced [116]. These findings indicate that, at least, in this case, apoptosis and inflammation share signaling pathways and others signaling factors [108].

Although apoptosis does not cause an inflammatory response, inflammation does sometimes occur, which is a matter that is not yet fully clarified. Some studies suggest that this may be due to how quick the apoptotic process occurs and how quick and efficient apoptotic cells are removed by phagocytes since, if this clearance is not effective, over time, the apoptotic cells develop a process known as secondary necrosis [93,97,117].

In case of excess of apoptosis or failure in cleaning cells in secondary necrosis, the membranes are degraded and become permeable allowing the escape of intracellular contents with proinflammatory molecules, thus stimulating the inflammatory response [97].

This inflammatory response to apoptotic cells should be viewed with optimism and properly explored, as it can be helpful in certain pathologies in which apoptosis has an important role, such as some viral infections and cancer. In these cases, the existence of an acute inflammation, associated with or in response to the abundance of apoptosis, may play an important role in promoting tissue healing/repair [109,115,118,119].

The presence of unremoved apoptotic cells has been linked to several different diseases, such as some infections or diseases with a marked inflammatory background, in the course of which Pathogen-Associated Molecular Patterns (PAMPs) and Damage-Associated Molecular Patterns (DAMPs) are detected and elicit leukocytes aggregates to destroy the pathogen and to start the repair process [120]. After, leukocyte recruitment ceases, and those that had

previously been recruited will be rapidly ingested by resident phagocytes and eliminated. In normal conditions, this clearance process can be remarkably efficient, and because of it, it is actually difficult to see apoptotic cells in organs and tissues. This failure can lead to a prolonged inflammatory response, causing apoptotic cells to accumulate locally, which often leads and has been observed in some diseases with an inflammatory background. This points to a tight link between cell death and inflammation [104,109,110,121].

In summary, apoptosis plays an important role in normal wound healing and tissue regeneration, and could fulfill the clinical need to prevent the harmful consequences of inflammation, for which apoptotic cells clearance is crucial. Finally, strategies to avoid defective clearance of apoptotic material could constitute new approaches for treating inflammatory or autoimmune diseases.

3.6. The p38MAPK Pathway and Apoptosis

Numerous studies have shown that activation of Mitogen-activated protein kinases (MAPK), particularly c-Jun N-terminal kinase (JNK) and p38, contributes to neuropathic pain pathology [43,122–124].

The activation of the p38MAPK signaling pathway plays an essential role in the generation and maintenance of neuropathic pain by regulating transcription, protein synthesis, receptor expression, and inducing apoptosis. The protein kinase A (PKA) is a key protein involved in neuropathic pain signaling, which by activating the p38MAPK intervenes in the apoptosis of spinal cord cell [46]. Studies have shown that PKA is closely related to inflammatory pain and bone cancer pain [124] but it is not clear its involvement in neuropathic pain caused by nerve damage. Authors hypothesized that PKA is involved in neuropathic pain by mediating spinal cord cell apoptosis through p38MAPK pathway activation, which culminates in a decrease in the antiapoptotic protein BCL2 and an increase in the proapoptotic proteins, TNF-alfa, IL1-beta, BAX, Caspase3 and 9 (Figure 1) [46]. Further, it has been suggested that the inhibition of either JNK or p38 may represent a potent clinical target for neuropathic pain management [46,122].

4. Apoptosis and Clinical Implications

The deregulation of cell death contributes to the development of numerous pathologies, namely cancer, ischemia, autoimmune, neurodegenerative diseases, and pain. The changes in cell death in these pathologies are not the same. There is a characteristic increase in some of them, and in others, there is a decrease in apoptosis.

Neuropathic pain is a kind of pain caused by damage to somatosensory nervous system mediated by multiple risk factors, including toxicity, surgery, and trauma. It reduces the quality of life of patients, being considered a public health problem worldwide. In this context, it becomes evident the need to deepen and improve the knowledge of the pathogenesis and pathophysiology in order to identify new biomarkers for the treatment of neuropathic pain.

4.1. Apoptosis and Neuropathic Pain

Nerve damage due to oxidative stress and mitochondrial dysfunction is a key pathogenic mechanism involved in chemotherapy-induced peripheral neuropathy (CIPN) [23,24,26–29,120]. On the other hand, TRP channels allow calcium to penetrate into nerve terminals. This can activate apoptosis mechanisms that result in nerve terminal destruction [33] in the skin which can cause long-term pain relief [33]. Further, cisplatin, oxaliplatin, and paclitaxel-induced mitochondrial oxidative stress, inflammation, cold allodynia, and hyperalgesia, through an increase in TRPA1 and TRPV4, leading to Ca^{2+} influx through direct channel activation or excessive production of oxidative stress and induction of apoptosis. The pain resulting from this Ca^{2+} overload is mediated through substance P (SP) and excitatory amino acid production. This chemotherapy-induced oxidative stress in DRG neurons that contribute to peripheral pain may be prevented with TRPA1 and TRPM8 antagonists such as reduced glutathione (GSH) and selenium [11,24,28,55,125,126].

Several studies have demonstrated that apoptotic activities in injured dorsal root ganglia increased after injury in different rat models [42,127,128] and that some agents could simultaneously suppress pain behavior and apoptotic activities [129,130]. However, there is no direct evidence that agents resulting in inhibition of apoptotic activities in the injured neurons could attenuate pain behavior [35].

4.2. Biomarkers and Circulating Mediators in Pain

Oxidative damage to peripheral neurons can cause damage to the myelin sheath, mitochondrial proteins, and other antioxidant enzymes [33]. Identification of levels of malondialdehyde, glutathione (GSH), superoxide dismutase (SOD), and activities of mitochondrial enzymes such as citrate synthase and ATP synthase can help monitor the course of peripheral neuropathy and response of neuropathy to the treatment [33].

Previous studies have indicated that proinflammatory cytokines, such as TNF-α, play an important role in the peripheral mediation of neuropathic pain and that altered dorsal root ganglion (DRG) function and the degree of DRG neuronal apoptosis are associated with spinal nerve injury. The level of TNF-alpha expressed in DRG after distal nerve crush injury is higher than that after proximal crush injury. The expression of DRG apoptosis in the distal crush injury was higher than in the proximal crush injury [42]. Nerve root crush injuries proximal to the DRG, such as those that may occur in the cauda equina, are less painful than spinal nerve crush injuries. The mechanism for these results may relate to DRG TNF-alpha expression and apoptosis [34,42,74,129,130].

Various stimuli, such as oxidative stress, TNF-α, and FAS antigen activation, can the phosphorylate serine/threonine kinase domain and activate Apoptosis signal-regulating kinase 1 (ASK1), an upstream protein in the p38 and JNK pathways. ASK1 has an important role in neuroinflammation during the induction and maintenance of chronic pain. Therefore, besides its potential as a pain biomarker, the inhibition of ASK1 may be a new therapeutic approach for neuropathic pain [131].

A study developed by Yuanzhi Peng and colleagues [62] shows that the upregulation of Ube2b, ubiquitin-conjugating enzyme E2B (Ube2b) as previously mentioned, ameliorates neuropathic pain by regulating potassium voltage-gated channel subfamily A member 2 (Kcna2) in primary afferent neurons, suggesting that this might be a novel biomarker for neuropathic pain-targeted therapies. This new knowledge opens perspectives for new therapeutic targets in various diseases, namely chronic pain [62].

Several recent studies have found that ubiquitin system failure is implicated in chronic pain, mainly including neuropathic pain (NP) and inflammatory pain [57]. Inflammatory pain is caused by an increase in the excitability of peripheral nociceptive fibers due to changes in ion channel activity caused by inflammatory mediators. Pain is triggered by normally innocuous stimuli during the inflammatory process and becomes chronic if the inflammation is not resolved. The mechanisms of NP are partly distinct from those of inflammatory pain, and potential therapeutic targets mediated by the ubiquitin system are different in NP and inflammatory pain [57].

4.3. Pain Therapy through Modulating Apoptosis Activities

Only one-third of patients receive pain relief from current analgesics, such as opiates, nonsteroidal anti-inflammatory drugs, local anesthetics, tricyclic antidepressants, and anticonvulsants, including carbamazepine and gabapentin. Therefore, it seems notable that recent developments in understanding the mechanisms that produce pain, either individually or collectively, have disclosed new potential therapeutic targets for developing more effective drugs [1].

The identification of antioxidant molecules with pleiotropic activity in other pathophysiological pathways involved in the CIPN could aid in the development and improvement of new therapies [120].

Isoflurane, a general inhalation anesthetic used for the induction and maintenance of general anesthesia, promotes PI3K/AKT activation, upregulates B-cell lymphoma

2 (Bcl-2)-associated X protein Bcl-2 expression levels, and reduces the expression levels of caspase 3 and caspase 8 in myocardial cells. Isoflurane is beneficial for pain attenuation and inhibits apoptosis in myocardial cells via the PI3K/AKT signaling pathway in mice during cardiac surgery [132]. Further, Masahiko Kawaguchi et al. (2004) showed that isoflurane reduced the early development of neuronal apoptosis in rats subjected to focal cerebral ischemia [133]. Other studies demonstrated that low concentrations of isoflurane attenuated the Aβ-induced reduction in Bcl-2/Bax ratio and led to a mild elevation in cytosolic calcium levels. However, these results are dose-dependent, suggesting that isoflurane may have dual effects on Aβ-induced toxicity (protection or promotion) [134]. JNK and p-JNK were found to continually increase with prolonged AOPPs stimulation. They were activated most significantly under the treatment of 50 μg/mL AOPPs–RSA. By using a JNK inhibitor (SP600125), DRG neurons were protected from AOPPs stimulation [34].

Paeoniflorin can not only significantly inhibit the activation of ASK1 and simulate the analgesic effect of ASK1 inhibitors, but also significantly inhibit the response of glial cells and neuroinflammation induced by CCI [131]. Acting in the same pathway, administration of a p38 inhibitor and a JNK inhibitor ameliorates neuropathic pain symptoms in rodent models [135,136].

Spare nerve injury (SNI) induces a significant increase in PKA expression in the spinal cord, and PKA is involved in neuropathic pain by activating the p38MAPK pathway to mediate spinal cord cell apoptosis [46].

Granulocyte colony-stimulating factor (G-CSF) is a cytokine that promotes the survival, proliferation, and differentiation of neutrophils. Recent studies have indicated that G-CSF also has non-hematopoietic functions and can potentially be used for the treatment of neuronal injury, including stroke and neurodegenerative disease. A clinical trial has shown that G-CSF may have a therapeutic effect on spinal neuropathic pain [137]. Several mechanisms and different signaling pathways are involved in the therapeutic role of G-CSF in neuropathic pain, including the upregulation of Mu-opioid receptor (MOR) and autophagic activity, through the suppression of BCL-2 expression in the injured nerve in the early phase [35].

Tetramethylpyrazine (TMP) attenuated neuropathic pain-associated hyperalgesia and neuronal apoptosis in the spinal dorsal horn, which was demonstrated by a decreased number of TUNEL-positive cells, upregulation in BCL-2 expression, and downregulation in caspase-3 expression in the spinal dorsal horn. These results suggest that TMP is beneficial for treating neuropathic pain by inhibiting apoptosis via the modulation of BCL-2 and caspase-3 proteins [138].

The crucial role of NF-kB in several pathologies that accompany pain provides evidence that targeting the NF-kB signaling cascade, including UPP, might have beneficial antinociceptive effects [64–66]. Several proteasome inhibitors are already approved to treat hematological neoplasias, such as multiple myeloma [139]. The generation of new proteasome inhibitors may represent a new pharmacotherapy for inflammatory pain [57]. Several studies show that UPP inhibitors can prevent inflammatory pain following injury and infection. The proteasome inhibitor MG132 can inhibit the activation of NF-κB, reversing the inflammatory pain [140].

Drugs that neutralize TNF have been developed and are used clinically to treat inflammatory and autoimmune diseases, such as rheumatoid arthritis, inflammatory bowel disease, and psoriasis [141]. However, despite their clinical success, the use of anti-TNF drugs is limited, but antibodies and bio-engineered ligands are currently in the preclinical and early clinical stages of development (such as infliximab, adalimumab, golimumab, and several biosimilars). Preclinical data obtained in different disease models show that the selective targeting of TNFRs has therapeutic potential and may be superior to global TNF blockade in several disease indications [141].

Moreover, studies have demonstrated that chemotherapy drugs, such as vincristine (Vin), could cause neuropathic pain and mitochondrial dysfunction. The mitochondrially targeted antioxidant, mitoquinone (MitoQ), can modify mitochondrial signaling, resulting

in beneficial effects on various diseases. Chen et al. investigated whether MitoQ could regulate vincristine-induced neuropathic pain and the underlying molecular mechanisms. They observed reduced expression of cleaved caspase 3 and BAX and increased protein levels of the antiapoptotic factor BCL-2 in the spinal cord of MitoQ-treated mice after vincristine stimulation. Further, MitoQ could alleviate vincristine-induced neuropathic pain by inhibiting oxidative stress and apoptosis via the amelioration of mitochondrial dysfunction (Figure 3) [142].

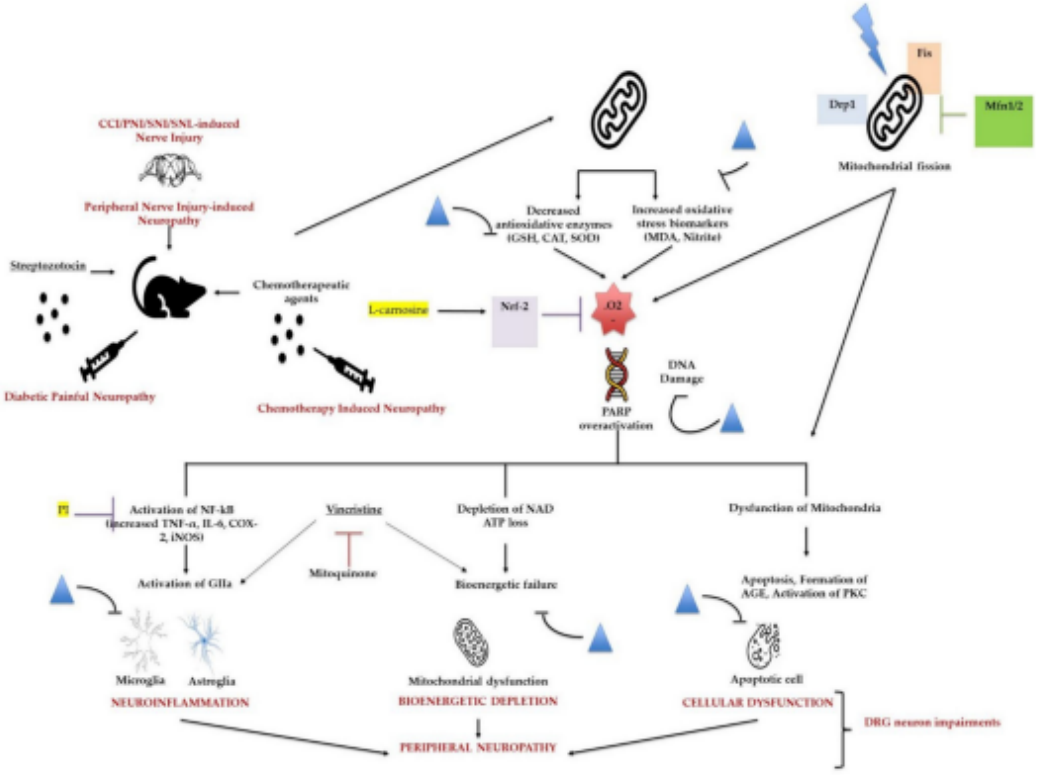

Figure 3. The effects of flavonoids and mitoquinone on neuropathic pain induced by vincristine. Flavonoids attenuate different peripheral neuropathic pain conditions by inhibiting or downregulating different neuroinflammatory, cellular, bioenergetic, and oxidative stress markers. Mitoquinone alleviates vincristine-induced neuropathic pain by improving mitochondrial dysfunction and inhibiting oxidative stress and apoptosis. Flavonoids; Nrf-2: nuclear factor erythroid 2–related factor 2; Drp-1: dynamin-related protein 1, Fis: mitochondrial fission protein; Mfn: mitofusin; .O2: reactive oxygen species/superoxide anion; PARP: poly(ADP-ribose) polymerase; NAD: nicotinamide adenine dinucleotide; AGE: advanced glycation end products; PKC: protein kinase C; PI: proteasome inhibitor.

Joseph et al. demonstrated that the caspase inhibitors could decrease the pain behaviors of rats that received chemotherapy (vincristine) and HIV therapy drugs [143]. Scholz et al. also showed that the caspase inhibitor could decrease pain behavior and the apoptosis of the dorsal horn of rats that received partial peripheral nerve injury [144].

There is also scientific evidence showing that antioxidants may be used preventively and therapeutically not only to reduce oxidative stress-related parameters, but also in-

flammatory response and pain in several diseases [11,145,146], and these effects could contribute to the reduction in apoptosis observed in neuropathic pain.

A randomized controlled study reported that L-carnosine exerted neuroprotective activity by significantly decreasing proinflammatory (NF-κB, TNF-α) and apoptotic (caspase 3) markers and increasing Nrf2 in colorectal cancer patients treated with oxaliplatin-induced PN. Therefore, the use of Nrf2 inducers in treating neuropathic pain in clinical settings holds great promise in the future [84].

The use of conventional analgesics, such as nonsteroidal anti-inflammatory drugs, opioids, tricyclic antidepressants, and anticonvulsants are reported to exhibit a wide spectrum of adverse effects, some of which limit their use for the treatment of neuropathic pain. Thus, the identification of new therapeutics with minimal side effects that can be used to alleviate neuropathic pain is important. For this purpose, the research and identification of analgesic molecules from natural sources could be considered. For instance, flavonoids and other antioxidants may attenuate neuropathic pain in different models summarized in Figure 3 [11,147].

Moreover, there are several ongoing preclinical studies targeting apoptosis mechanisms in order to treat chronic pain (Table 1). However, future studies are needed to translate these results into clinical practice.

Table 1. Some preclinical/clinical studies with drugs/compounds involved in apoptosis that could potentially treat pain.

Drugs	Administration and Time of the Experiment	Main Results	Model	Localization of Changes	References
Apocynin	Intrathecal lumbar injection; studied 42 days after injury	↓ NP, mechanical sensitivity	Animal model of SCI—Rat	Behavioral testing only	[145]
Corilagin	IP injection for 4 weeks after injury	↓ Oxidative stress, NF-KB, ↓ TNF-α, IL6 ↓ Caspase 3, ↓ Pain	Animal model of SCI—Rat	Spinal cord	[148]
Curcumin	IP injection for 28 days after injury	↓ IL-2, NF-KB, TNF-α ↓ Pain	Animal model of SCI—Rat	Spinal cord	[149]
Deferoxamine	IP injection on the first day after injury, followed by once a week during 12 weeks	↓ Oxidative stress ↓ Iron overload markers ↓ Pain	Animal model of SCI—Rat	Hind limb sensory cortex, hippocampus, thalamus	[150]
Epigallocatechin 3-gallate	IV infusion for 36 h after 4 h post-lesion (acute) and after 12 months (chronic)	↓ Glial activity ↓ NP ↓ Lesion size area ↑ Number of spinal neurons	Animal model of SCI—Rat	Spinal cord	[151]
Geraniol	IV infusion 6h after lesion; evaluation at 4 weeks and 8 weeks	↓TNF-α, ↓NMDAR1 ↓ Caspase 3, glial activation ↑ HO-1, Nrf2. ↓ NP	Animal model of SCI—Rat	Spinal cord	[152]
Acupuncture	Once a day on 31 to 33 d after lesion	↓ Inflammation inhibitor, ROS scavenger, ↓ ERK inhibitor, ↓ p38MAPK inhibitor ↓ NP	Animal model of SCI—Rat	Spinal cord (L4–L5)	[153]
Progesterone	Daily sc injections (6 h, 1 day, 28 days after injury)	↓ NF-KB, COX-2, iNOS ↓ NP	Animal model of SCI—Rat	Spinal cord (L4–L5)	[154]
SB203580 (P38MAPK antagonist)	35 days Intraspinal injection in L4/L5 space	SCI ↑-P38MAPK ↓ P38MAPK, NP with antagonist	Animal model of SCI—Rat	Spinal cord (dorsal horn neurons)	[155]
Resiniferatoxin (molecular neurosurgery)	One time	↓ TRPV1 ↓ NP, cancer pain	Clinical trial phase 2		[156]

Table 1. *Cont.*

Drugs	Administration and Time of the Experiment	Main Results	Model	Localization of Changes	References
Hypericum perforatum	Gastric gavage for 4 weeks	↓ TRPV1, TRPM8, ROS ↓ NP	Animal model of CCI injury of the sciatic nerve—Rat	Sciatic nerve and DRG	[157]
VAS2870 (NADPH oxidase inhibitor) Roscovitine (CdK5 activity inhibitor)	24 h 1 h	↓NOX1, NOX2/NADPH oxidase complex, TNF-α ↓Inflammatory pain	Primary culture of mouse nociceptive neurons	Trigeminal ganglia, DRG, and HEK293 cells	[83]
Isoflurane (low concentration)	One time (6 h or 30 min)	↑ PI3K/AKT activation ↑Cytosolic calcium levels	Cell line Mice primary neurons	Mice primary neurons H4 human neuroglioma cells	[134]
SP600125 (JNK inhibitor)	One time	AOPP ↑ JNK, and ↑ DRG neurons apoptosis by activating caspase 3 and PARP-1. ↓ by SP600125	Cell culture Rat model	Rat DRG neurons	[34]
Paeoniflorin (ASK1 inhibitor)	One time	↓ ASK1, glial cells, and neuroinflammation, ↓ ↓ ↓ TNF-α, IL-1	Animal model of CCI injury of the sciatic nerve—Rat	Spinal dorsal horn	[131]
G-CSF	One time	↑ MOR, autophagic activity, BCL-2	Clinical trial		[137]]
Tetramethylpyrazine	One time (3, 7 and 14 days post-surgery)	↑ BCL-2 ↓ Caspase-3 expression ↓ NP	Animal model of CCI injury of the sciatic nerve—Rat	Superficial spinal dorsal horn (L4–L5)—laminae I and II	[138]
MG132 (Proteasome inhibitor)	One time	↓ Activation of NF-κB ↓ Inflammatory pain	Rat model of rheumatoid arthritis and SCI	DRG	[140]
Mitoquinone	One time	↓ Caspase-3 and BAX ↑ BCL-2 ↓Vincristine-induced neuropathic pain	Vincristine-induced neuropathic pain model in ICR mice Primary neuron culture of DRG	Lumbar (L4–L5) DRG Spinal cord (L4–L5) Sciatic nerve	[142]

↓ Decrease, ↑ Increase. DRG: dorsal root ganglia; COX-2: cyclooxygenase-2, HO-1: heme oxygenase-1, IL: interleukin, iNOS: inducible NO synthase, ERK: extracellular-signal-regulated kinase, MMP: matrix metalloproteinase, mTOR: mammalian target of rapamycin, NF-κB: nuclear factor-κB, Nrf2: nuclear factor erythroid 2-related factor 2, NP: neuropathic pain, PI3K: phosphoinositide 3-kinases, p38MAPK: p38 mitogen-activated protein kinases, ROS: reactive oxygen species, TNF-α: tumor necrosis factor-alpha. SCI: spinal cord injury, CCI: chronic constriction injury, IP: intraperitoneal, IV: intravenous, SC: subcutaneous, NMDAR1: N-methyl-D-aspartate receptor 1, NP: neuropathic pain, SCI: spinal cord injury, DRG: dorsal root ganglion, AOPP: advanced oxidative protein products, ASK1: apoptosis signal-regulating kinase 1, G-GCF: granulocyte-colony-stimulating factor (G-CSF).

5. Conclusions and New Perspectives

The deregulation of apoptosis is involved in the development of several pathologies, and recent evidence suggests that apoptosis may be involved in chronic pain, namely neuropathic pain.

Neuropathic pain is a chronic pain condition caused by primary damage or dysfunction of the nervous system, but the fine molecular mechanisms have not yet been fully elucidated.

Previous studies have indicated that NF-kB, proinflammatory cytokines such as TNF-α, and oxidative stress play an important role in peripheral mediation of neuropathic pain through apoptosis modulation. Further, the degree of DRG neuronal apoptosis has recently been associated with spinal nerve injury via caspase signaling and/or PKA activation through the p38MAPK pathway, generating and maintaining neuropathic pain.

The adoption of measures to prevent and protect mitochondrial function may constitute a promising therapeutic strategy to alleviate or prevent chronic pain conditions [79] since mitochondria are a primary source of cellular ROS [120]. Pharmacological interventions aimed at maintaining normal mitochondrial function may be an alternative therapeutic approach to the direct free radical scavengers for the treatment of CIPN [120]

Medications that can modulate proinflammatory cytokine expression, the NF-kB pathway, and apoptosis, such as TNF-alpha modulators, G-CSF, proteasome inhibitors, and flavonoids, among others, have the potential to treat neuropathic pain [35]. However, the timing of administration, dose, and indications for neuropathic pain treatment still need to be validated by clinical studies [35].

Further, the inhibition of apoptosis via the modulation of BCL-2 and caspase-3 is beneficial for the treatment of neuropathic pain [138].

The involvement of apoptosis in pain and the clinical relevance of this knowledge in the potential discovery of new biomarkers and therapeutic targets can result in the development of more effective and targeted drugs to treat chronic pain, particularly neuropathic pain.

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Opioids and constipation therapy in the last week of life

Their impact on patients, caregivers, and the location of death

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Abstract

The use of opioids to control pain at the end of life may cause constipation, a symptom that can negatively influence the well-being of patients and caregivers. The main aim of this study was to evaluate the impact of constipation on symptomatic control and patients' overall quality of life at this stage. A particular focus was placed on opioids. We also intended to investigate whether constipation and caregiver fatigue is related to the place of death (hospital vs home). The approach of 121 patients followed in 2021 in their last week of life by a home team specialized in palliative care was analyzed in an observational, retrospective, non-interventional study. The patients were followed up for an average of 39.7 days. A total of 82.6% wished to die at home, which occurred in 74% of the cases. The constipation prevention protocol reduced constipation by 55.1%. It seems that morphine is more related with constipation and tapentadol seems to reduce constipation induced by opioids. Patients tended to die in hospitals when their caregivers were exhausted; however, it was not possible to determine a cutoff point using the Zarit scale, which was used to assess caregiver burden. Constipation in the last week of life does not seem to influence the well-being of patients or their caregivers significantly and the individualization of intensive treatment of constipation is needed. Different opioids have different probabilities of causing adverse effects such as constipation. Future special support mechanisms can be created and activated for the most tired caregivers to avoid exhaustion and promote death at home, if that is the patient's will.

Abbreviation: POS = palliative outcome scale.

Keywords: caregivers, end of life care, palliative care, opioid, pain management, constipation

1. Introduction and objectives

More than 40 million people will need specialized palliative care every year around the world.[1] Pain is one of the most common symptoms in patients in need of palliative care[2,3] and is most frequent at the end of life.[3] The use of opioids to control pain in these situations is not only necessary but also ordinary. Opioid therapy has constipation as a side effect,[4] which is one of the most common symptoms in patients with palliative needs, particularly at the end of life.[3] Keeping patients at home, in comfort, and accompanied by their loved ones is the best option, but it is necessary to create and support home teams to attend to them in all suffering dimensions.

The existence of home palliative care teams requires trained caregivers.[5,6] Therefore, mental and physical health of caregivers is one of the main concerns of palliative care teams.[6,7]

Since opioids are often used to treat pain and constipation inevitably appears as a side effect,[4] the main aim of this study was to assess whether constipation and its management have a significant impact on the control of other symptoms and on the quality of life of patients and caregivers in the last week of life. We also intended to evaluate the leading causes of patient death in palliative care and their place of death with this follow-up, aiming to assess whether the latter contradicts the trend of death in hospitals.

The authors have no funding and conflicts of interest to disclose.

All data generated or analyzed during this study are included in this published article [and its supplementary information files].

The authors declare that they have Ethical Comission authorizations from Group of Health Centers Gaia and Faculty of Medicine of University of Porto. This study was approved by the Ethics Committee of Faculty of Medicine of University of Porto and North Regional Health Administration of Portugal.

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Key points

- Opioids are widely used in the last week of life to control symptoms, such as pain and dyspnea.
- Constipation is one of the most common side effects of opioids.
- Constipation is one of the most common symptoms in the last days of life.
- It is known that constipation is challenging to treat in patients needing specialized palliative care.
- Several studies have reported different outcomes regarding the probability of opioid-causing constipation.
- There have been no studies on the importance of treating constipation in the last week of life.
- We do not know the importance of palliative care home teams in the last week of life for patients and caregivers and their wishes.
- We did not have any information about caregivers' exhaustion and the probability of dying at the hospitals.
- In this study, we determined the importance of treating constipation when patients have symptoms during the last days of life. It seems that it is not as important as we thought and has no influence on the quality of life of patients and caregivers.
- We identified some advantages of specialized palliative care at home.
- We could also identify opioids with a higher probability of causing constipation and opioids with a lower probability of causing that symptom.
- We could not identify a cutoff point using the Zarit scale, but patients died at the hospital and not at home (as they wanted to) when caregivers were exhausted (with Zarit scale points of >20).

As secondary objectives, we aimed to evaluate whether there are opioids with less potential for constipation while maintaining the same effectiveness in controlling pain, and whether the protocol used for prevention and treatment of constipation, following the recommendations in the literature,[8] has been effective in the control of this symptom. Finally, it also intends to identify a cutoff point between caregiver burden and place of death.

2. Materials and methods

This study collected data from the last days of life of 121 patients who died in 2021, followed by a home palliative care team in northern Portugal. This was an observational, retrospective, non-interventional study, using medical and nursing records made in the last 8 days of life of patients followed by the same home palliative care team.

Data collection was carried out by recording in a protected Microsoft Excel sheet, without transferring the identity of the patients. Following the General Data Protection Regulation, the files were eliminated after the end of the study and publication of the results.

The following variables were included: age, sex, primary diagnosis, time elapsed from the start of follow-up by the team until death (in days), place of death (home, hospital, or other), constipation for >3 days in the last 8 days of life, type of constipation (mechanical or functional), opioid and dose used, and laxative use.

The recommended therapy to prevent constipation included the use of first-line osmotic laxatives such as macrogol and lactulose when patients had been prescribed opioids on a fixed regimen and/or had a history of constipation.[4,8,9] If >3 days of constipation, the patient started a bowel-stimulating laxative (such as bisacodyl).[8] After >4 days of constipation, they began Senna and/or contact laxatives, such as sodium lauryl sulfate or sodium docusate.[8] If the situation did not improve, a manual assessment was performed on the sixth or seventh day, and as there was no possibility of obstruction (we didn´t have any patient with mechanical constipation), a cleansing enema was performed.[4,8]

Whenever possible, the Edmonton scale was used to assess symptoms,[10] the palliative outcome scale (POS) to assess patients' quality of life,[11] and the palliative performance scale to measure functionality.[12] Caregiver burden was assessed using the Zarit scale.[13]

Qualitative variables were presented as absolute and relative frequencies and quantitative variables with means and standard deviations. The significance level for rejecting the null hypothesis was set at (α) ≤.05. Fisher exact test, Mann–Whitney U test, logistic regression, and receiver operating characteristic curves were used. The normality of distribution was analyzed using the Shapiro–Wilk test and the homogeneity of variances using the Levene test. The qualitative variables were transformed into dummy variables. The Hosmer–Lemeshow goodness-of-fit test was used for the goodness of fit for the logistic regression.

Statistical analysis was performed using Statistical Package for the Social Sciences software (version 28.0 for Windows, IBM Corp., Armonk, NY).

3. Results

In 2021, 198 patients were monitored by the same home palliative care team, of which 121 died (61%). Patients who died had a mean age of 78.1 years, between a minimum of 34 years and a maximum of 103 years, and 69 males (67%). They were all bedridden and totally dependent on daily living activities, with functionality measured by palliative performance scale[12] between 10% and 30%.

The home palliative care team followed patients for an average of 39.7 days, but cancer patients were followed up for a mean of 47 days (20.5% more follow-up time).

As shown in Figure 1, cancer (66.9%, n = 81) was the most frequent cause of death in this period, followed by neurological diseases (14%), organ failure (14%), and other causes (4.2%), which included autoimmune diseases, chronic pain syndromes, and frailty syndrome in the elderly.

Of the 121 patients, 100 registered their domicile as the intended place of death (82.6%). As observed in Figure 2, approximately 74% of the patients died at home (n = 90), 15% in the hospital (n = 18), and 11% in institutions (residential care homes for the elderly and continuing care unit).

Of the 121 patients who died during the study period, no patient had mechanical constipation, and 95.9% (n = 116) had opioids prescribed in the last week of life; of these, 53.7% (n = 65) did not have constipation during this week.

On average, patients had a score of 28.9 in the last week of life on the Edmonton scale[10] and a POS score[11] of 17.5.

Regarding the degree of caregiver burden, the mean score obtained using the Zarit scale was 40, and the main caregivers thus presented a moderate to severe burden on average. Regarding the impact of constipation in the last week of life on caregivers, it was observed that they were less tired (average Zarit score of 38).

However, no significant relationships were found between place of death, caregiver burden, or Edmonton scale score and constipation (P > .05), as shown in Table 1.

Then, a logistic regression was performed with the variables Edmonton, Zarit and constipation as independent or predictive variables and the local variable of death as the explained or dependent variable. The difference between the model plus the independent variables and the model with the constant alone

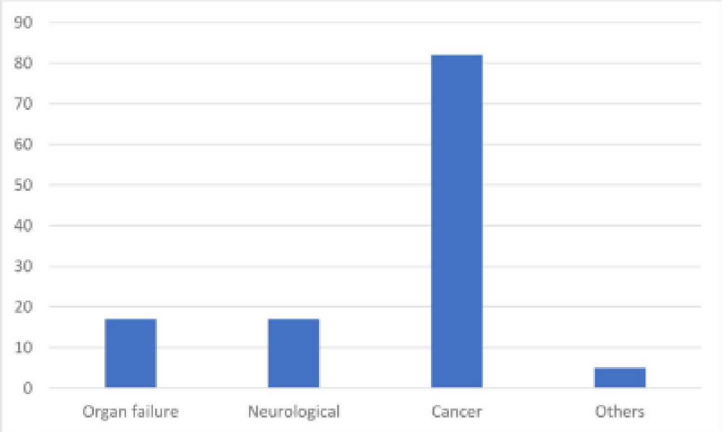

Figure 1. Causes of death (absolute numbers). Causes of death: cancer (n = 81), neurological diseases (n = 17), organ failure (n = 17), and others (n = 6).

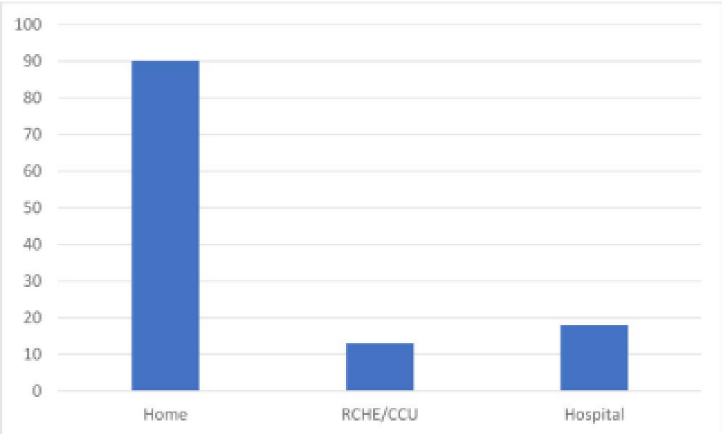

Figure 2. Place of death (absolute number). Place of death: home (n = 90), residential care homes for the elderly/continuing care units (ERPI/UCC [n = 13], and hospital [n = 18]).

Table 1

Bivariate analysis.

	Home	Hospital	Sig.
Obstipation			.531
No	51 (77.3%)	15 (22.7%)	
Yes	39 (70.9%)	16 (29.1%)	
Zarit[14] (M; SD)	20.72 (7,49)	17.64 (14,53)	.713
Edmonton[12] (M; SD)	30.29 (14.03)	24.93 (10.85)	.140

M = mean, SD = standard deviation, Sig. = significance.

was not statistically significant (χ^2 (3) = 5.809, P = 121). The model explains 7.8% of the decrease in uncertainty in identifying the place of death. The Hosmer–Lemeshow goodness-of-fit test was χ^2 (8) = 21.174, P = .007, indicating a poor fit to the data. The independent variables were not significant predictors of place of death (P > .05), as shown in Table 2.

Regarding the use of opioids, 95 patients were prescribed morphine, of which 42 were also on another major opioid, 25

Table 2

Logistic regression (place of death).

		B	SE	Wald	Sig.	Exp (B)	95% CI for Exp (B) Lower	Upper
Step	Edmonton	−.031	.020	2.369	.124	.970	.933	1.008
	Zarit	−.019	.024	.645	.422	.981	.935	1.028
	Obstipation	.628	.479	1.718	.190	1.874	.733	4.792
	Constant	−.335	.683	.241	.624	.715		

B = coefficient for the constant in the null model, CI = confidence interval, Exp (B) = exponentiation of the B coefficient, SE = standard error, Sig. = significance. Wald = Wald test.

were on fentanyl, 20 were on tapentadol, 13 were on buprenorphine, and 5 were on tapentadol and buprenorphine.

The occurrence of constipation in relation to the use of different opioids varied, as shown in Table 3.

On average, 41.5% of the patients who had constipation in the last week of life were taking opioids.

The significant relationships between the variables under study and constipation are listed in Table 4.

Patients treated with morphine seem to have more constipation and patients treated with tapentadol seem to have less constipation, according with other studies[14–17] that had less patients.[16,17] In patients treated with the remaining opioids, there were no significant differences in developing constipation.

Regarding caregiver burden, it was not possible to define a cutoff point for the place of death. The area under the receiver operating characteristic curve of sensitivity and specificity between the place of death and caregiver burden was not statistically significant (P = .713), there was no reason to find a cutoff point. However, all patients who died in the hospital during the study period had caregivers with a Zarit score[13] ≥20.

4. Discussion and conclusions

Data related to the primary diagnosis and cause of death showed that most patients had cancer (67.8%). Despite this, non-cancer patients represent the majority (approximately 70%) of patients in need of palliative care, worldwide.[1] The present results suggest that patients with non-cancer diseases have increased difficulty in accessing palliative care, which is reinforced by the fact that the follow-up time of patients with

Table 3

Frequency of constipation in the last week of life with the different opioids.

Opioid	Patients with constipation (%)
Morphine	46 (48.4%)
Morphine + another opioid	17 (40.5%)
Fentanyl	11 (44%)
Buprenorphine	6 (46.2%)
Tapentadol	6 (30%)
Tapentadol + buprenorphine	2 (40%)

Table 4

Significant relationship between constipation and the other variables under study.

	No N	No %	Yes N	Yes %	Sig.
Morphine	23	51.1%	22	48.9%	.577
Tapentadol_50_100	3	42.9%	4	57.1%	.701
Tapentadol100_200	0	0.0%	1	100.0%	.455
Tapentadol200_400	0	0.0%	0	0.0%	–
Buprenorphine17.5_35	3	60.0%	2	40.0%	1.000
Buprenorphine52.5	0	0.0%	1	100.0%	.455
Buprenorphine70	1	25.0%	3	75.0%	.329
Fentanyl12.5	5	35.7%	9	64.3%	.160
Fentanyl25	8	47.1%	9	52.9%	.602
Fentanyl50	2	22.2%	7	77.8%	.077
Fentanyl75	2	40.0%	3	60.0%	.658
Fentanyl100	0	0.0%	1	100.0%	.455
Lactulose	1	7.7%	12	92.3%	.001***
Macrogol	3	75.0%	1	25.0%	.625
Macrogol + Sodium bicarbonate	13	34.2%	25	65.8%	.003**
Sodium picosulfate	2	10.5%	17	89.5%	.001***
Isacodyl	6	24.0%	19	76.0%	.001***
Microlax	1	5.0%	19	95.0%	.001***
Senna	2	18.2%	9	81.8%	.022*
Cleansing enema	0	0.0%	3	100.0%	.091
Manual	0	0.0%	4	100.0%	.040*

N = absolute number of patients, Sig. = significance.

* $P \leq .05$.

** $P \leq .01$.

*** $P \leq .001$.

cancer is 20.5% longer than the mean follow-up time of all patients. Overall, these facts suggest that cancer patients are referred earlier to palliative care and with that, the opportunity for greater satisfaction with the response to their needs and the care provided.

The fact that a significant number of patients expressed a wish to die at home (100 out of 121), which happened to the majority in this study, reinforces the benefits of palliative care at home since, in Portugal, only 29.6% of patients die at home.[18] However, only 8.9% referred to the hospital as their preferred place of death in a country where 61.7% of people die in these institutions.[18]

There are published papers of varying quality, as reviewed by Hofmeister and colleagues, assessing different components of palliative care in the home interventions and measuring different outcomes.[19] To be meaningful to patients, these interventions need to be consistently evaluated with outcomes that matter to the patients and caregivers,[19] as we presented in this work.

Morphine appeared to be the opioid that is more related with constipation. In contrast, tapentadol appears to be less associated with constipation.

Constipation occurs in approximately 97% of patients on chronic opioid therapy.[9] The protocol followed by the home palliative care team significantly reduced constipation by 55.1% in the studied patients.[4,8,9]

Constipation in the last week of life does not seem to influence the well-being of patients, which is noticeable by the lack of statistical significance between Edmonton and POS of constipated and non-constipated patients. Constipation also had no statistically significant influence on caregiver burden. Thus, intensive therapies to control this symptom in the last week of life should be considered only in specific cases in which the general condition worsens.

Although we cannot present a significant cutoff point between the caregiver burden measured by the Zarit scale and the place of death with high sensitivity and specificity, it is evident that patients tend to die in the hospital when the caregiver is most exhausted.

In this sense, it is important to develop special support mechanisms for caregivers who present with symptoms and signs of tiredness to fulfill the wishes of both (patients and caregivers) to enable their end of life at home.

This work has limitations, not only because it is a unicentric study, but also because of the small sample size. However, to our knowledge, this is the first study to interpret the relationship between the use of opioids, laxatives, constipation, caregiver burden, and the location of death. We can now understand that some symptoms, such as constipation, may have a more conservative approach at the end of life, ensuring the well-being of both patients and caregivers. More similar studies involving larger numbers of patients are needed to obtain additional data and solid conclusions.

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Platelet membrane proteins as pain biomarkers in patients with severe dementia

biomedicines

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Article

Platelet Membrane Proteins as Pain Biomarkers in Patients with Severe Dementia

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Abstract: Pain is one of the most frequent health problems, and its evaluation and therapeutic approach largely depend on patient self-report. When it is not possible to obtain a self-report, the therapeutic decision becomes more difficult and limited. This study aims to evaluate whether some membrane platelet proteins could be of value in pain characterization. To achieve this goal, we used 53 blood samples obtained from palliative patients, 44 with non-oncological pain and nine without pain. We observed in patients with pain a decrease in the percentage of platelets expressing CD36, CD49f, and CD61 and in the expression levels of CD49f and CD61 when compared with patients without pain. Besides that, an increase in the percentage of platelets expressing CD62p was observed in patients with pain. These results suggest that the levels of these platelet cluster differentiations (CDs) could have some value as pain biomarkers objectively since they are not dependent on the patient's participation. Likewise, CD40 seems to have some importance as a biomarker of moderate and/or severe pain. The identification of pain biomarkers such as CD40, CD49f, CD62p and CD61 can lead to an adjustment of the therapeutic strategy, contributing to a faster and more adequate control of pain and reduction in patient-associated suffering.

Keywords: chronic pain; pharmacology; platelets; biomarkers; palliative care

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1. Introduction

The International Association for the Study of Pain (IASP) defines pain as an "unpleasant sensory and emotional experience associated with, or resembling that associated with, actual or potential tissue damage" [1]. Pain is multifactorial, with multiple pathways involved [2], which explains why it is a multidimensional experience that can significantly impair an individual's quality of life [3]. Pain can be classified according to tissue damage (nociceptive pain), nerve damage (neuropathic pain), and altered pain modulation (nociplastic pain) [3].

Pain characterization is essential for the correct approach for patients with chronic pain, starting with the assessment of pain intensity [4]. The proper characterization is dependent on patient self-report; pain multidimensional hetero-assessment scales can be

used as an alternative, especially for dementia patients with and/or patients that cannot characterize their pain [5–7]. However, as pain is a sensory and emotional experience, it is subjective, and its expression is primarily determined by the perceived intensity of the painful sensation [5].

Preclinical and clinical studies have investigated the hypothesis that biomarkers may also be used to identify and quantify pain. Findings from a preclinical study show that inflammatory pain and neuropathic pain have different biomarkers [8], but most studies do not correlate with pain duration or intensity. Further studies are needed to gain insights into pain biomarkers to enhance pain management practices improving patient care, especially for those who suffer from severe cognitive decline or dementia and are unable to express themselves.

Platelet heterogeneity and subpopulations may suggest distinct biological roles for different platelet subpopulations and may be useful in evaluating inherited or acquired platelet disorders and platelet function in health and disease [9]. Besides their role in hemostasis, thrombosis, and wound healing, platelets are now known to play major effector activities in several additional functions, including inflammatory reactions and innate immune responses [9]. Further, platelets are the closest and most accessible peripheral neuronal-like cellular system likely to provide a wealth of information about neuronal functioning [10].

Since discovered by Giulio Bizzozero in 1882 [11], platelets have been exploited for their clinical value. Platelet surface receptors, such as CD62p (P-selectin) and CD41 (GPIIb-IIIa), have also been quantified as markers of the activation state of platelets [12]. The extravascular activation of platelets may contribute to nociceptor excitation and pain since platelets store and, upon stimulation, release potential allogenic substances such as serotonin, histamine, and precursor molecules of bradykinin [13,14].

Identifying blood and platelet pain biomarkers has been advanced as the next great tool for pain identification and characterization, allowing tailored treatments [15,16].

Few studies relate pain with platelet activation, as pain inhibition occurs with platelet antiaggregants [17–20]. However, there are other studies on specific situations, for instance, knee osteoarthritis or post-teeth extraction pain, where platelet-rich plasma (PRP) or fibrin-rich platelets (PRF) may have a significant analgesic effect [21–24]. However, the role of peripheral blood platelets membrane proteins as markers for pain evaluation and characterization is not yet clarified [25,26].

Here, we evaluated the levels of membrane proteins, namely receptors related to several recognized functions of platelets, such as recognition, adhesion, aggregation, activation, inflammation, and immune modulation. In this context, the levels of the glycoprotein IV (CD36), the adhesion molecules, integrin α6 (CD49f), integrin β3 (CD61) and p-selectin (CD62p), the complement activation inhibitor protein (CD59) and the TNFα family receptor CD40 were evaluated in palliative patients platelets.

The main aim of this study is to evaluate whether some known platelet membrane proteins could be of value in pain characterization and can be used as non-invasive pain biomarkers in patients where we cannot rely on their self-report, particularly patients with advanced dementia.

2. Materials and Methods

For this study, we collected individual and clinical data and peripheral blood samples from 53 palliative patients with non-oncological diseases, followed by a specialized palliative care team between 1 September and 31 December 2021.

This is an observational, analytic, transversal, non-interventional study using medical and nursing records on chronic pain patients.

The Ethics Committees of the Faculty of Medicine of the University of Porto and the North Regional Health Administration of Portugal approved the research procedures, and the study was conducted following the Declaration of Helsinki. Before enrollment, participants or their legal representatives provided informed consent for participation. The

international ethical guidelines of confidentiality, the anonymity of personal data, and the abandonment option were followed.

For the collection of individual and clinical data, we consulted the records in the individual clinical files, after which they were registered in a protected Microsoft Excel sheet. To identify each patient, an alphanumeric code was used, thus keeping the identity confidential since only the researcher knows it. Following the European General Data Protection Regulation (GDPR), these electronic files will be deleted after the end of the study and the publication of the results. Data were collected regarding the following variables: age, sex, type and intensity of pain, opioid and other analgesics, such as nonsteroidal anti-inflammatory drugs (NSAIDs) and acetaminophen, and doses in use. We also noted whether the patient had pain control at the moment of blood sample collection.

We also collected information regarding the presence of a diagnosis of dementia and its type. For patients that had severe dementia, we used the Pain Assessment in Advanced Dementia Scale (PAINAD) [27] to identify and distinguish those who probably have uncontrolled pain, and we separated PAINAD values (under 5, between 5 and 7 and 8 to 10), to evaluate a possible clinical correlation of these PAINAD values and mild, moderate or severe pain, respectively. For patients who could self-report their pain, we used the pain numeric scale [28].

For the purpose of analyzing the expression of the platelet biomarkers, we selected the following: glycoprotein IV (CD36), integrin α6 (CD49f), integrin β3 (CD61), and p-selectin (CD62p), the complement activation inhibitor protein (CD59) and TNFα family receptor CD40, that we analyzed in absolute and relative concentration.

We proceeded to the ROC curve analysis to evaluate the significance of the area under the curve and if we have a cut-off point for CD40.

2.1. Platelets Membrane Proteins Evaluation by Flow Cytometry

The platelet phenotyping was performed in freshly prepared platelet suspensions. None of the participants were recently transfused. Briefly, platelets were separated by centrifuging citrated blood specimens at 120× *g* for 20 min at room temperature (RT). Then, the platelet-rich plasma was collected into a tube, and the platelets were washed using 2 mL of wash buffer (BD cell wash). Next, 1×10^6 platelets were incubated with the monoclonal antibodies anti-CD36 conjugated with FITC (BD Pharmingen, BD Biosystems, San Diego, CA, USA), anti-CD49f conjugated with PE conjugate (BD Pharmingen, BD Biosystems), anti-CD61 conjugated with PerCP-Cy5.5 (BD Pharmingen, BD Biosystems), anti-CD62 conjugated with APC (BD Pharmingen, BD Biosystems), anti-CD59 conjugated with BV421(BD Horizon, BD Biosystems), and anti-CD40 conjugated with BV510 (BD Horizon, BD Biosystems) for 15 min at RT in the dark, according to manufacture instructions. Then, cells were washed twice with FACS flow (BD Biosystems) by centrifugation at 300× *g* for 5 min and immediately analyzed in a FACS Canto II flow cytometer (BD Biosystems). At least 50,000 events were collected using FACS DIVA software (BD Biosystems), and the results were analyzed through Infinicyte software (Cytognos, Salamanca, Spain). The results are expressed in the percentage of cells expressing each protein marker and as mean fluorescence intensity (MIF).

2.2. Statistical Analysis

Statistical analysis was performed using the SPSS software (Statistical Package for the Social Sciences, version 28.0 for Windows, IBM Corp., Armonk, NY, USA). For the statistical analysis of data, we used measures of descriptive statistics (absolute and relative frequencies, means, and respective standard deviations) and inferential statistics. The significance level for rejecting the null hypothesis was set at $(\alpha) \leq 0.05$. We used the Pearson correlation coefficient, Fisher's test, Chi-squared test of independence, Student's *t*-test for independent samples, Mann–Whitney U test, and Kruskal–Wallis test. The normality of distribution was analyzed with the Shapiro-Wilk test, and the homogeneity of variances was analyzed with Levene's test. We analyzed the Chi-squared assumption that there

should not be more than 20% of cells with expected frequencies inferior to 5. In those situations where this assumption could not be satisfied, we used the Chi-squared test with the Monte Carlo simulation.

The following variables were included: age, sex, type of pain, the intensity of pain, opioid and dose used, and other analgesics, such as nonsteroidal anti-inflammatory drugs (NSAIDs) and paracetamol, and absolute (MIF) and relative concentration levels of CD36, CD49f, CD61, CD62p, CD59, and CD40. We also included the type of dementia (because most of the patients had this condition) and controlled pain time for patients diagnosed with chronic pain, but their pain is controlled at the moment of blood collection. For patients that had severe dementia, we used the Pain Assessment in Advanced Dementia Scale (PAINAD) [27] to identify uncontrolled pain. Other variables that could have a relationship with our findings were also included, such as renal function (using the Cockcroft–Gault formula [29], body mass index (BMI), functionality (using the Karnovsky scale [30]), and nutritional status (using Mini-Nutritional Assessment scale [31]).

2.3. *Inclusion Criteria*

All patients under clinical follow-up from a palliative care specialized team from the North region of Portugal with non-oncological diseases.

All patients or their legal representatives provided informed consent for participation.

3. Results

We selected 95 patients. However, five patients or their legal representatives refused to participate in this study and 20 of them were in their last days of life, and it was decided not to conduct a blood collection in this clinical condition. We could not collect blood samples from 17 patients, as they were too fragile, with hypovolemia and bad venous accesses.

So, we collected samples from a total of 53 patients with an average age of 74.8 years old, a minimum of 29 and a maximum of 98 years old; most were female [n = 39 (73.6%)]. Forty-four patients suffered from pain, and nine had no pain. We had no patients with known autoimmune diseases (inflammatory bowel disease, multiple sclerosis, psoriasis, or other of these conditions).

Among the 44 patients with chronic pain, 38 had severe dementia. We could not evaluate the type of pain and intensity in these patients. Therefore, we used PAINAD to distinguish those who probably have uncontrolled pain (PAINAD $\geq$ 5, present in 32 patients), and we separated PAINAD values (under 5, between 5 and 7 and 8 to 10) because in the clinical evaluation, there was a correlation between these PAINAD values and the possibility of having mild, moderate or severe pain, respectively. Among the 44 patients with pain, 19 were under opioid treatment (15 with dementia and four without dementia).

We had six patients with pain and without dementia and nine without pain and without dementia (controls), as shown in Table 1.

Table 1. Characterization of the Study Population.

Total of Patients: 53		**Patients with Pain and Dementia (n = 38)**	**Patients with Pain and without Dementia (n = 6)**	**Patients without Pain and without Dementia (Controls) (n = 9)**
Gender	**Male**	29% (n = 11)	50% (n = 3)	0
	Female	71% (n = 27)	50% (n = 3)	100%
Average age (years)		84.1	62.5	44.7
PAINAD	**<5**	15.8% (n = 6)	NA	NA *
	5–7	73.7% (n = 28)	NA	NA
	8–10	10.5% (n = 4)	NA	NA
Average numeric pain scale		NA	4.3	0

Table 1. *Cont.*

Total of Patients: 53		Patients with Pain and Dementia (n = 38)	Patients with Pain and without Dementia (n = 6)	Patients without Pain and without Dementia (Controls) (n = 9)
Type of Pain	Nociceptive	39.5% (n = 15)	50% (n = 3)	NA
	Neuropathic	2.6% (n = 1)	16.7% (n = 1)	NA
	Mixed	57.9% (n = 22)	33.3% (n = 2)	NA
Type of Dementia	Vascular	36.8% (n = 14)	NA	NA
	Alzheimer	36.8% (n = 14)	NA	NA
	Mixed	7.9% (n = 3)	NA	NA
	Other	18.4% (n = 7)	NA	NA
Under opioid treatment		39.5% (n = 15)	66.7% (n = 4)	NA

* NA: Not Applicable.

In the platelets of patients with chronic pain, we observe a statistically significant decrease in the percentage of platelets expressing CD36, CD49f, and CD61, but the decrease in the expression levels of these biomarkers was only observed for CD49f and CD61, compared with those patients without pain (Table 2). An increase in the percentage of platelets expressing CD62p was detected when compared with patients without pain (p = 0.002) (Table 2).

Table 2. Platelets Membrane Proteins in Patients with and without Chronic Pain.

	Patients without Pain and without Dementia Controls (n = 9)	Patients with Pain (n = 44)	
	M ± SD	M ± SD	p
CD36 (%)	98.04 ±1.97	91.54 ± 12.30	0.004 **
CD36 (MIF)	6475.64 ± 2081.58	5506.31 ± 2168.98	0.228
CD49f (%)	99.16 ± 0.35	95.31 ± 10.79	0.037 *
CD49f (MIF)	5385.83 ± 855.09	4540.24 ± 969.50	0.021 *
CD61 (%)	99.37 ± 0.18	95.28 ± 10.76	0.026 *
CD61 (MIF)	16561.81 ± 1404.83	13571.25 ± 4064.78	0.009 **
CD62P (%)	8.47 ± 4.87	22.62 ± 23.65	0.002 **
CD62P (MIF)	760.75 ± 114.10	1357.76 ± 2606.10	0.499
CD59 (%)	4.76 ± 3.03	3.45 ± 5.67	0.507
MIF CD59 (MIF)	1556.62 ± 509.90	1771.15 ± 858.56	0.478
CD40 (%)	0.15 ± 0.09	0.09 ± 0.17	0.289
CD40 (MIF)	1476.08 ± 352.14	1456.12 ± 501.07	0.911

MIF—Mean Fluoresce intensity; * $p \leq 0.05$, ** $p \leq 0.01$. F: median intensity fluorescence; M: mean; SD: standard deviation; p: significance.

While the differences observed in CD36, CD49f and CD61 are age and sex independent, the difference observed in CD62p were more accentuated in men (about three times higher) comparatively with the observed in women (Table 3). When we compared the platelets phenotype between patients with dementia with those without dementia, we did not observe any statistically significant difference. Further, the relationship between the type of pain and the type of dementia is also not statistically significant (Table 3).

Table 3. Gender And Membrane Protein Platelets.

	Female (n = 39)	**Male (n = 14)**	
	M ± SD	**M ± SD**	***p***
% CD36	92.89 ± 10.89	92.58 ± 13.25	0.899
MIF CD36	5392.26 ± 2050.79	6662.28 ± 2330.91	0.090
% CD49f	96.35 ± 8.80	95.18 ± 12.86	0.445
MIF CD49f	4617.57 ± 978.43	4986.02 ± 1057.26	0.291
% CD61	96.38 ± 8.80	95.13 ± 12.83	0.272
MIF CD61	14465.44 ± 3481.59	13172.91 ± 4992.24	0.525
% CD62P	**13.76 ± 17.43**	**39.24 ± 24.50**	**0.006 *****
MIF CD62P	829.68 ± 261.73	2549.57 ± 4691.50	0.251
% CD59	3.74 ± 5.35	3.59 ± 5.20	0.839
MIF CD59	1643.84 ± 790.28	2000.70 ± 815.41	0.130
% CD40	0.09 ± 0.10	0.14 ± 0.28	0.939
MIF CD40	1471.59 ± 505.76	1423.23 ± 362.89	0.899

*** $p \leq 0.001$; MIF: median intensity fluorescence; M: mean; SD: standard deviation; p: significance; bold: values statistically significant.

As we can observe in Table 4, patients under opioids, when compared with patients that did not receive opioids, present a statistically significant decrease in the percentage of platelets expressing CD36 (88.52% vs. 95.58, $p = 0.026$) and in the expression levels of CD49f (4254.22 vs. 4995.90, $p = 0.012$), CD61 (12643.79 vs. 15128.72, $p = 0.029$) and CD59 (1345.35 vs. 1975.92, $p = 0.008$).

Table 4. Comparative Analysis Of Membrane Platelets Proteins Between Patients Receiving And Not Receiving Opioids Medication.

	No Opioids (n = 25)	**Opioids (n = 19)**	
	M ± SD	**M ± SD**	***p***
% pos CD36	**95.58± 8.38**	**88.52± 14.02**	**0.026 ***
MIF CD36	6007.26 ± 2091.56	5211.73 ± 2246.28	0.228
% pos CD49f	98.82 ± 2.39	91.78 ± 14.55	0.070
MIF CD49f	**4995.90± 850.84**	**4254.22± 1065.23**	**0.012 ***
% pos CD61	98.87 ± 2.40	91.73 ± 14.51	0.077
MIF CD61	**15128.72± 3014.28**	**12643.79± 4616.87**	**0.029 ***
% pos CD62P	21.08 ± 22.70	17.93 ± 21.35	0.290
MIF CD62P	1483.69 ± 2983.98	863.37 ± 388.08	0.242
% pos CD59	2.94 ± 3.33	4.90 ± 7.28	0.597
MIF CD59	**1975.92± 886.94**	**1345.35± 444.10**	**0.008 ****
% pos CD40	0.07 ± 0.08	0.15 ± 0.23	0.742
MIF CD40	1485.36 ± 467.55	1420.60 ± 490.21	0.458

* $p \leq 0.05$, ** $p \leq 0.01$; MIF: median intensity fluorescence; M: mean; SD: standard deviation; p: significance; bold: values statistically significant.

Regarding the type of pain and its relationship with biomarkers under study, we found statistically significant differences, as shown in Table 5. A decrease in the expression levels of CD62p in the platelets of patients with nociceptive pain was observed compared with patients presenting mixed pain (723.52 vs. 1953.63, $p = 0.002$) (Table 5).

Table 5. Membrane Proteins Platelets In Patients Suffering From Nociceptive Pain Or Mixed Pain.

	Nociceptive (n = 18)	**Mixed (n = 26)**	
	M ± SD	**M ± SD**	***p***
% pos CD36	91.17 ± 11.90	92.52 ± 10.84	0.762
MIF CD36	4751.72 ± 1640.23	5952.76 ± 2468.46	0.118
% pos CD49f	94.47 ± 12.16	97.46 ± 5.19	0.789
MIF CD49f	4206.69 ± 995.35	4859.81 ± 890.59	0.056
% pos CD61	94.37 ± 12.13	97.45 ± 5.15	0.762
MIF CD61	12507.53 ± 5215.25	14025.16 ± 3190.66	0.682
% pos CD62P	13.89 ± 14.69	31.73 ± 28.35	0.117
MIF CD62P	**723.55 ± 184.49**	**1953.63 ± 3679.90**	**0.002 ****
% pos CD59	4.49 ± 6.59	3.05 ± 5.46	0.789
MIF CD59	1570.69 ± 826.36	1963.51 ± 937.17	0.274
% pos CD40	0.16 ± 0.25	0.04 ± 0.02	0.145
MIF CD40	1530.59 ± 601.99	1466.89 ± 448.98	0.986

** $p \leq 0.01$, MIF: median intensity fluorescence; M: mean; SD: standard deviation; p: significance; bold: values statistically significant.

The clinical history (other diseases) of the patients does not seem to interfere with platelet biomarkers.

With regard to pain intensity, we observed a significant relationship with the percentage of platelets expressing CD40. In fact, the percentage of platelets expressing CD40 is significantly higher in patients with moderate-severe pain (considering PAINAD ≥5) compared with those with mild pain (0.18% vs. 0.02%, $p = 0.047$), as it is shown in Table 6.

Table 6. Membrane Proteins Platelets In Patients Suffering From Mild Pain And With Moderate Or Severe Pain.

	Mild Pain (PAINAD < 5)	**Moderate or Severe Pain (PAINAD 5-10)**	
	M	**M**	***p***
% pos CD36	90.34 ± 18.24	92.88 ± 9.64	0.563
MIF CD36	5635.97 ± 2538.02	5424.41 ± 2158.15	0.859
% pos CD49f	99.19 ± 0.66	95.94 ± 9.29	0.625
MIF CD49f	4310.17 ± 1200.38	4549.61 ± 949.86	0.723
% pos CD61	99.16 ± 0.66	95.90 ± 9.27	0.625
MIF CD61	10900.50 ± 4611.48	13915.31 ± 3929.31	0.059
% pos CD62P	16.62 ± 21.39	23.77 ± 24.53	0.690
MIF CD62P	3966.84 ± 7066.90	918.62 ± 383.06	0.533
% pos CD59	1.25 ± 1.49	3.90 ± 6.08	0.282
MIF CD59	1699.58 ± 1043.69	1793.88 ± 856.69	0.533
% pos CD40	**0.02 ± 0.02**	**0.10 ± 0.18**	**0.047 ***
MIF CD40	1202.00 ± 232.79	1509.31 ± 524.97	0.207

* $p \leq 0.05$,MIF: median intensity fluorescence; M: mean; SD: standard deviation; p: significance; bold: values statistically significant.

We used receiver operating characteristic curve (ROC) analysis to verify if CD40 could be a peripheral biomarker of pain intensity. We proceed to the ROC curve analysis to evaluate the significance of the area under the curve and if we have a cut-off point for this

biomarker. The area under the curve is statistically significant, 0.777, with $p = 0.049$, as shown in Figure 1 and Table 7.

The ideal cut-off point corresponds to 0.025 (sensitivity 74.2%, specificity 80%, positive predictive value = 95.8%, negative predictive value = 33.3%).

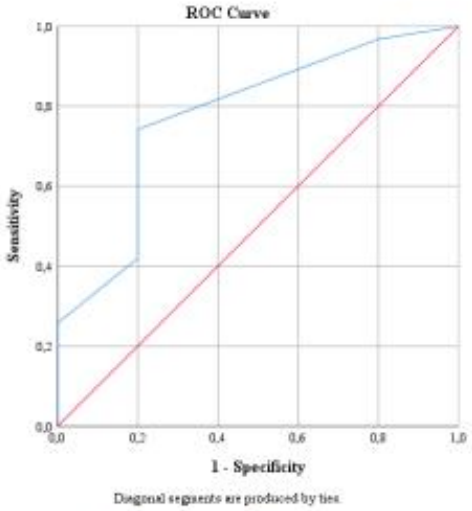

Figure 1. ROC curve of CD 40 as a biomarker of moderate or severe pain.

Table 7. ROC Analysis Data for CD40.

Area	SE [a]	Asymptotic p [b]	Asymptotic 95% Confidence Interval	
			Lower Bound	Upper Bound
0.777	0.114	0.049	0.555	1.000

[a]: Standard Error; [b]: Significance.

4. Discussion

In patients with severe dementia, we cannot rely on self-report, and the characterization of the pain is difficult. The identification of biomarkers could be a valuable solution to enable targeted medical treatment. In this study, we find that CD36, CD49f, percentage of CD49f, CD61, percentage of CD61, and CD62p can be considered as platelet biomarkers of pain, CD62p as a platelet biomarker for nociceptive pain and CD40 as a platelet biomarker for moderate-severe pain.

The hypothesis that biomarkers may be used to identify and quantify pain was investigated in several preclinical and clinical studies. A preclinical study showed that inflammatory and neuropathic pain have different biomarkers [8]. Further investigations provided mixed results. For example, cystatin C levels in the cerebrospinal fluid appear to be a predictive marker for postherpetic neuralgia in patients with varicella-zoster virus and a pain marker in women experiencing labor pain. However, it is not correlated with pain duration or intensity. Investigations into potential biomarkers for chest pain showed that cardiac markers used to aid in the diagnosis and prognosis of cardiac disease correlate with tissue damage rather than with pain [8]. Further studies are needed to gain insights into biomarkers for pain to enhance pain management practices.

Platelet receptors are important for their normal functioning as they either activate platelets or act as adhesion molecules interacting with the damaged endothelium, other platelets, and leukocytes. Besides the platelet role in hemostasis, they also have a role in inflammation, antimicrobial activity, angiogenesis, tumor growth, and metastasis. In

the absence of their receptors, platelets are unable to perform these functions. Some of the well-recognized platelet receptors are integrins, leucine-rich repeats receptors, selectins, tetraspanins, transmembrane receptors, prostaglandin receptors, lipid receptors, immunoglobulin superfamily receptors, tyrosine kinase receptors, and other platelet receptors [32]. The present study focused on membrane glycoproteins, such as CD36, CD49f, CD61, CD62p, CD59, and CD40.

CD36 or Glycoprotein IV is a member of the class B scavenger receptor family of cell surface proteins, a multiligand pattern recognition receptor that interacts with a large number of structurally dissimilar ligands, including long chain fatty acid (LCFA), advanced glycation end products (AGE), thrombospondin-1, oxidized low-density lipoproteins (oxLDLs), high density lipoprotein (HDL), phosphatidylserine, apoptotic cells, beta-amyloid fibrils (fAβ), collagens I and IV, and Plasmodium falciparum-infected erythrocytes [33].

CD49f is an adhesion molecule, namely α6-integrin 1, associated with inflammation towards the regulation of differentiation, adhesion, and migration of human mesenchymal stem cells [34]. CD61 is the integrin β3, a glycoprotein that plays a role in platelet aggregation and also as a receptor for fibrinogen, fibronectin, von Willebrand factor and vitronectrin [35]. CD62p or P-selectin is a membrane protein that redistributes to the plasma membrane during platelet activation and degranulation and mediates the interaction of activated endothelial cells or platelets with leukocytes [36]. CD59 is the complement activation inhibitor protein that binds to complement components C5 and C9 and prevents the polymerization of C9, which is required for the formation of the membrane attack complex (MAC) [37]. CD40 is a receptor of the TNFα family, being a cytokine produced by many cells, and was originally identified by its cytotoxic effects. In addition to inducing cell death in some types of cells, it also elicits a wide range of physiological responses, such as inflammation, cell proliferation, and differentiation [38]. Further, circulating biomarkers of platelet activation, including soluble CD40 and CD62p, have also been studied as a strategy to monitor the efficacy of combination antiretroviral therapy (cART) in patients infected with HIV [39].

Platelet surface receptors have also been quantified as markers of the activation state of platelets, and platelet activation is increased in dementia [40]. Both platelet CD62p (P-selectin) expression and CD41 (GPIIb-IIIa) complex activation are significantly elevated in Alzheimer's Dementia (AD) patients [41]. CD41 is a platelet activation marker in dementia, and the increase in CD41 complex expression in platelets was associated with faster cognitive decline in AD [41]. However, there is an overwhelming lack of additional clinical studies. Another platelet receptor, CD62p, is present on activated platelet membranes [42] and promotes platelet adhesion and thrombin formation [43]. Increased levels of CD62p were found in circulation in AD patients, but there was no significant change in the membrane-bound [12,38]. The soluble form of CD62p was also significantly elevated in HIV patients not under cART, compared with those cART-treated and with healthy control groups, suggesting its role in monitoring combination antiretroviral therapy [39].

We already know that there is platelet activation in pain, especially inflammatory pain (Barkai et al., 2019; Beurling-Harbury and Schade, 1989), but we are still unaware of all the pathophysiology and molecular biology involved [19]. The identification of CD36, CD49f, percentage of CD49f, CD61, percentage of CD61, and CD62p as platelet biomarkers of pain was statistically significant. These new markers must be considered in the process of obtaining pain biomarkers in peripheral blood.

For patients with well-controlled pain, in comparison with those with non-controlled pain, we did not find statistically significant differences regarding laboratory data, including patients with well-controlled pain for seven or more days. These data are relevant because we could expect a reduction in platelet activation with pain control, considering that the half-life of platelets is seven days. However, it may mean that some underlying mechanisms and causes of pain may be equally active, and there is only pain desensitization.

In opioid-treated patients presenting a better control of pain, there is less expression of biomarkers such as CD36, CD49f, CD61, and CD59. These data are in agreement with the literature on the effect of opioids on platelets, particularly in reducing their activity [44–46].

The sample was too small to assess more types of pain in addition to nociceptive and mixed pain. However, CD62p has been shown to be a reliable marker of nociceptive pain. Although it has not yet been specifically studied in this regard, the role of p-selectin is already known and has been linked to inflammatory pain [47,48].

CD40 (TNFα receptor family) was identified as a potential biomarker of moderate or severe pain intensity. The association of a biomarker with pain severity has not yet been established, although there are several references to platelet markers and disease severity [49–51]. In addition to the biomarkers of the existence or not of pain, which can be a powerful aid in the therapeutic approach of patients, the identification of biomarkers that allow the characterization of pain, in this case by intensity, can allow the therapeutic adjustment as quickly as possible, reducing suffering and minimizing the adverse effects of drugs.

Further, and besides the involvement of platelets in several diseases the presented markers, such as CD40, in autoimmune diseases such as inflammatory bowel disease, multiple sclerosis, psoriasis, or other autoimmune diseases, we had no patients with these diseases. Other diseases from the known medical history of these patients (such as hypertension) did not influence the platelet markers, according to what we found in the literature [9,10,12,17,26].

This is one of the largest studies on pain biomarkers that we know of, and it is the only study comparing patients with non-oncological pain with specific platelet biomarkers. However, the sample is small, and further studies are needed considering these markers to confirm their viability as pain markers and CD40 as a marker of moderate-severe pain.

In vulnerable and dependent patients, in whom we cannot rely on self-report, the identification of pain biomarkers such as CD40 can lead to an adjustment of the therapeutic strategy, according to the WHO ladder [4], contributing to a faster and more adequate control of pain and reduction in associated suffering.

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Monocytes in the characterization of pain in palliative patients with severe dementia – a pilot study

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Monocytes in the Characterization of Pain in Palliative Patients with Severe Dementia—A Pilot Study

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Abstract: In assessing and managing pain, when obtaining a self-report is impossible, therapeutic decision-making becomes more challenging. This study aimed to investigate whether monocytes and some membrane monocyte proteins, identified as a cluster of differentiation (CD), could be potential non-invasive peripheral biomarkers in identifying and characterizing pain in patients with severe dementia. We used 53 blood samples from non-oncological palliative patients, 44 patients with pain (38 of whom had dementia) and 0 without pain or dementia (controls). We evaluated the levels of monocytes and their subtypes, including classic, intermediate, and non-classic, and characterized the levels of specific phenotypic markers, namely CD11c, CD86, CD163, and CD206. We found that the relative concentrations of monocytes, particularly the percentage of classic monocytes, may be a helpful pain biomarker. Furthermore, the CD11c expression levels were significantly higher in patients with mixed pain, while CD163 and CD206 expression levels were significantly higher in patients with nociceptive pain. These findings suggest that the levels of monocytes, particularly the classic subtype, and their phenotype markers CD11c, CD163, and CD206 could serve as pain biomarkers in patients with severe dementia.

Keywords: monocytes; biomarkers; chronic pain; dementia; pain characterization

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1. Introduction and Objectives

Pain is a multidimensional experience that can significantly impair a patient's quality of life. It can be classified according to the underlying cause, including nociceptive pain resulting from tissue damage, neuropathic pain caused by nerve injury, and nociplastic pain resulting from altered pain modulation [1]. As multiple factors influence pain, it is essential to characterize it accurately to treat chronic pain patients with success [2].

Patient self-reporting is regularly used to characterize pain. However, it is subject to individual interpretation, which can be a problem for proper pain characterization. To overcome this subjectivity, multidimensional hetero-assessment scales have been developed, particularly for patients with dementia or others unable to characterize their pain [3–5].

However, this approach is insufficient for this type of patient, and there is an urgent need for a more objective characterization using non-invasive biomarkers.

Routine peripheral blood parameters, including white blood cell counts and mean platelet volume (MPV), have been identified as potential diagnostic, prognostic, and predictive response markers in inflammatory and central nervous system diseases, such as cerebral hemorrhage and dementia in elderly patients [6]. Further, blood plasma, blood cells, skin fibroblasts, and peripheral blood vessels were considered diagnostic biomarkers for Alzheimer's disease [7]. Our previous studies show that some platelet cluster of differentiation (CD) levels could be valuable as pain biomarkers, particularly for pain subtype classification and pain intensity characterization [8].

Monocytes are a type of white blood cell crucial in promoting and resolving inflammation and are implicated in several inflammatory diseases, such as cardiovascular diseases, diabetes, obesity, and metabolic syndrome [9,10]. There are three main subsets of monocytes, classical (~85%), non-classical (~10%), and intermediate (~5%), which are characterized by their expression levels of CD14 and CD16 [10,11]. These subsets play distinct roles in homeostasis, inflammation, and various diseases such as rheumatoid arthritis, complex regional pain syndrome, cancer, tuberculosis, and HIV [11–14]. Studies have reported an increased proportion of intermediate monocytes in various inflammatory states, including cardiovascular disease [9,10]. Circulating monocytes correlate with several diseases that cause pain [15–17]. However, statistical robustness for this correlation with pain and pain characterization is lacking.

This study aims to assess the levels of monocyte subsets (classical, non-classical, and intermediate) [10,16] and the transmembrane proteins CD11c, CD86, CD163, and CD206, which are related to various monocyte functions, including immune response during infection or inflammation [18–21] in non-oncological palliative patients. Furthermore, this study aims to assess the potential of these membrane proteins in characterizing pain and serving as non-invasive biomarkers for patients who cannot self-report their pain, especially those with advanced dementia.

2. Results

We initially selected 95 patients. However, the legal representatives of five patients refused to participate in this study. Seventeen patients were excluded from blood sample collection due to their fragile condition, hypovolemia, and difficult venous access. Also, 20 patients were in their last days of life, and we decided to avoid blood collection in this clinical condition.

We obtained blood samples from 53 patients with an average age of 74.8 years old, a minimum of 29, and a maximum of 98 years old. Most of the patients were female [n = 39 (73.6%)]. Nine patients had no pain, and forty-four suffered from chronic pain. We had no patients diagnosed with autoimmune diseases such as inflammatory bowel disease, multiple sclerosis, psoriasis, or other conditions.

Of the 44 patients with chronic pain, 38 also had severe dementia, making assessing their pain intensity and type difficult. As a result, we relied on the PAINAD scale to identify patients who likely had uncontrolled pain. Patients with PAINAD $\geq$ 5 (present in 32 patients) are those who probably have uncontrolled pain [22]. Among the 44 patients with pain, 19 were receiving opioid treatment (15 with dementia and 4 without dementia). We saw no significant differences between the prevalence of pain in males and/or females. When we compared the monocyte phenotype between different clinical features such as renal function, hepatic function, nutritional status, and biotype (weight, height), we did not observe any statistically significant difference.

We had six patients with pain without dementia and nine patients without pain and without dementia that were considered controls, as shown in Table 1.

Table 1. Characterization of the study population.

Total of Patients: (n = 53)		Patients with Pain and Dementia (n = 38)	Patients with Pain without Dementia (n = 6)	Patients without Pain and without Dementia (Controls) (n = 9)
Gender	Male	29% (n = 11)	50% (n = 3)	0
	Female	71% (n = 27)	50% (n = 3)	100%
Average age (years)		84.1	62.5	44.7
PAINAD scale	<5	15.8% (n = 6)	NA	NA *
	5–7	73.7% (n = 28)	NA	NA
	8–10	10.5% (n = 4)	NA	NA
Average numeric pain scale		NA	4.3	0
Type of Pain	Nociceptive	39.5% (n = 15)	50% (n = 3)	NA
	Neuropathic	2.6% (n = 1)	16.7% (n = 1)	NA
	Mixed	57.9% (n = 22)	33.3% (n = 2)	NA
Type of Dementia	Vascular	36.8% (n = 14)	NA	NA
	Alzheimer	36.8% (n = 14)	NA	NA
	Mixed	7.9% (n = 3)	NA	NA
	Other	18.4% (n = 7)	NA	NA
Under opioid treatment		39.5% (n = 15)	66.7% (n = 4)	NA

* NA: Not Applicable. PAINAD: Pain Assessment in Advanced Dementia.

2.1. Monocyte Characterization in Patients with and without Chronic Pain

In patients with pain, we observed a significant increase in the percentage of total monocytes (10.27% vs. 4.19%, $p = 0.025$), in particular in the classic monocytes (91.1% vs. 85.3%, $p = 0.003$), and we saw a decrease in the intermediate (5.7% vs. 3.1%, $p = 0.008$) and non-classic monocytes (5.53% vs. 2.79%, $p = 0.011$) compared with patients without pain (Figure 1 and Table 2).

When we evaluated, in each monocyte subset, the expression of CD11c, CD163, and CD206, we did not find any statistical differences in these markers in intermediate and non-classic monocytes between patients with and without pain. However, in total and classic monocytes, we found statistically significant differences in CD206 and CD163, when comparing these biomarkers with the presence of pain, as shown in Table 2. We discovered a significant rise in the proportion of monocytes expressing CD206 in patients experiencing pain, as opposed to those without pain (59.36% vs. 14.08%, $p = 0.047$). On the other hand, we detected, in these patients, a decrease in the expression levels of CD206 (469.01 vs. 534.29 MFI, $p = 0.019$), CD163 (2378.85 vs. 3061.99 MFI, $p = 0.05$), and in the ratio of CD163/CD206 (4.81 vs. 9.5, $p = 0.004$) in monocytes (Table 2). In classical monocytes, all of the same protein receptors are decreased in patients with pain compared to those without pain (percentage of cells expressing CD163—95.6 vs. 88.0 MFI, $p = 0.01$, CD206—11.2% vs. 6.7%, $p = 0.039$, and ratio CD163/CD206—11.1% vs. 6.5%, $p = 0.038$ and expression levels of CD206 were 503.4 vs. 472.9 MFI, $p = 0.021$).

No differences between controlled and uncontrolled pain patients were observed in the studied biomarkers.

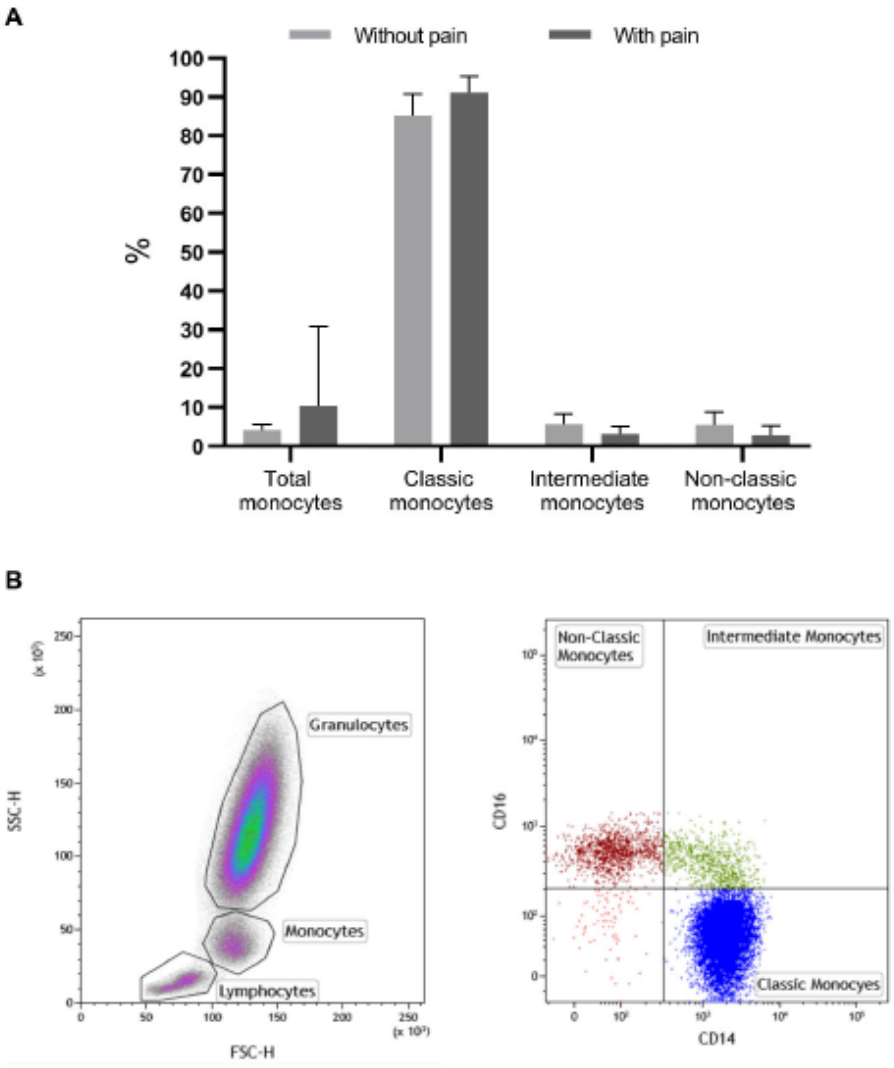

Figure 1. Percentage of monocytes and their subsets, classic, intermediate, and non-classic in patients without and with pain (**A**). In (**B**), there are representative dot-plots of the identification of monocytes based on SSC/FSC (right dot-plot) and of monocyte subsets (left dot-plot): classic monocytes (CD14+/CD16−); intermediate monocytes (CD14+/CD16+); non-classic monocytes (CD14−/CD16+). %—percentage; significant values: total monocytes (10.27% vs. 4.19%, $p = 0.025$); classic monocytes (91.1% vs. 85.3%, $p = 0.003$); intermediate monocytes (5.7% vs. 3.1%, $p = 0.008$); non-classic monocytes (5.53% vs. 2.79%, $p = 0.011$); *p*—significance.

Table 2. Monocyte biomarkers and pain.

	Without Pain (n = 9)	**With Pain (n = 44)**	
	M ± SD	**M ± SD**	***p***
Monocytes			
%	**4.19 ± 1.45**	**10.27 ± 20.5**	**0.025 ***
% CD11c	88.52 ± 7.23	294.67 ± 923.03	0.181
MIF 11c	816.98 ± 313.70	820.86 ± 303.38	0.503
% CD86	32.69 ± 12.27	705.21 ± 3073.33	0.130
MIF 86	634.20 ± 20.58	608.65 ± 143.02	0.850
% CD163	85.63 ± 6.36	115.35 ± 150.62	0.487
MIF 163	**3061.99 ± 1033.45**	**2378.85 ± 1840.89**	**0.050 ***
% CD206	**14.08 ± 7.80**	**59.36 ± 230.35**	**0.047 ***
MIF 206	**534.29 ± 54.31**	**469.01 ± 14.66**	**0.019 ***
% 11c/86	31.76± 12.10	99.96 ± 352.47	0.123
% 163/206	**9.5 ± 5.7**	**4.8 ± 5.6**	**0.004 ****
Classical monocytes			
%	**85.3 ± 5.4**	**91.1 ± 4.2**	**0.003 ****
% CD11c	91.9 ± 8.3	93.6 ± 9.4	0.250
MIF 11c	740.6 ± 286.2	825.4 ± 260.7	0.182
% CD86	30.1 ± 12.3	21.7 ± 13.7	0.070
MIF 86	592.1 ± 13.7	593.1 ± 23.8	0.781
% CD163	**95.6 ± 6.3**	**88.0 ± 9.4**	**0.010 ****
MIF 163	3185.1 ± 1119.8	2526.7 ± 1799.5	0.079
% CD206	**11.2 ± 6.9**	**6.7 ± 6.3**	**0.039 ***
MIF 206	**503.4 ± 48.6**	**472.9 ± 89.8**	**0.021 ***
% 11c/86	29.0 ± 12.1	21.0 ± 13.0	0.091
% 163/206	**11.1 ± 6.8**	**6.5 ± 6.1**	**0.038 ***
Intermediate			
%	**5.7 ± 2.6**	**3.1 ± 2.0**	**0.008 ****
% CD11c	98.9 ± 1.0	98.4 ± 3.1	0.770
MIF 11c	1769.1 ± 601.1	1743.8 ± 551.8	0.947
% CD86	78.0 ± 9.5	68.5 ± 22.5	0.376
MIF 86	813.7 ± 90.3	853.4 ± 103.1	0.174
% CD163	88.1 ± 9.6	79.7 ± 13.9	0.064
MIF 163	2397.9 ± 1125.5	2843.9 ± 3818.2	0.174
% CD206	43.7 ± 17.6	35.7 ± 15.5	0.328
MIF 206	537.4 ± 70.1	548.4 ± 185.5	0.682
% 11c/86	77.8 ± 9.6	68.4 ± 22.4	0.362
% 163/206	41.5 ± 18.0	33.2 ± 15.3	0.229

Table 2. *Cont.*

	Without Pain (n = 9)	With Pain (n = 44)	
	M ± SD	M ± SD	*p*
Non-classical			
%	**5.53 ± 3.25**	**2.79 ± 2.50**	**0.011 ***
% CD11c	78.1 ± 30.4	78.7 ± 26.2	0.812
MIF 11c	1612.8 ± 623.4	1713.7 ± 548.2	0.518
% CD86	58.8 ± 26.0	64.2 ± 26.9	0.391
MIF 86	791.0 ± 82.1	854.3 ± 121.9	0.129
% CD163	14.6 ± 12.4	12.6 ± 7.6	0.771
MIF 163	813.05 ± 196.72	885.05 ± 327.74	0.947
% CD206	17.0 ± 16.3	11.4 ± 9.2	0.187
MIF 206	634.7 ± 203.5	572.9 ± 347.5	0.099
% 11c/86	58.7 ± 26.0	64.1 ± 26.9	0.383
% 163/206	4.1 ± 4.7	3.8 ± 4.9	0.561

M—mean; SD—standard deviation * $p \leq 0.05$ ** $p \leq 0.01$. %—percentage; MFI—mean fluorescence intensity; *p*: significance. The statistically significant differences are highlighted in bold.

2.2. Monocytes and Gender

When comparing the biomarkers and the gender of patients, we found significant differences between the percentage of cells expressing CD86 and the ratio CD11c/CD86, which have higher values in men (30.0 vs. 20.5 MFI, $p = 0.026$ and 28.37 vs. 20.1 MFI, $p = 0.047$).

2.3. Type of Pain and Monocyte Biomarkers

As presented in Table 3, we only detected statistically significant differences in the percentage of non-classic monocytes. The percentage was higher in patients with nocioceptive pain (3.72% vs. 1.82%, $p = 0.037$) than in patients with mixed pain.

However, we found statistically significant differences when comparing types of pain and CD11c, CD163, and CD206.

The percentage of monocytes expressing CD11c is significantly higher in patients with mixed pain, while in patients with nociceptive pain, we observed an increase in the expression levels of CD163 and CD206 and the ratio CD163/CD206.

Concerning the monocyte subpopulations, there is an increase in the expression levels of CD11c in all subpopulations of monocytes in patients with mixed pain compared with those with nocioeptive pain, in which the percentage of monocyte expression of this receptor is only changed in classic and intermediate monocytes (Table 3). Moreover, in non-classic monocytes, only CD11c is altered.

In patients with nociceptive pain, the expression levels of CD206 are significantly higher in classical and intermediate monocytes compared with patients with mixed pain (classic—478.16 vs. 469.28 MFI, $p = 0.016$; intermediate—575.94 vs. 517.74 MFI, $p = 0.041$), while CD163 is only changed in classic monocytes.

Therefore, while the levels of CD163 and CD206 are significantly higher in nociceptive pain, the levels of CD11c are significantly higher in mixed pain.

With multilogistic regression, we did not find any statistically relevant differences, so we did not perform multinominal regression. The specificity of the model was 88.2% and the sensibility was 77.8%. The model's discriminative capacity was exceptional (0.905), with the area under the curve being statistically significant, as is shown in Figure 2.

Table 3. Type of pain and monocyte biomarkers.

	Nociceptive (n = 18)	**Mixed (n = 20)**	
	M ± SD	**M ± SD**	***p***
Granulocytes	71.75 ± 12.42	73.00 ± 11.06	0.808
Linfocytes	20.51 ± 10.86	637.36 ± 1843.35	0.395
Monocytes			
%	5.48 ± 2.26	15.77 ± 29.48	0.301
% CD11c	**83.16 ± 20.92**	**539.46 ± 1332.28**	**0.002 ****
MIF 11c	743.56 ± 165.92	870.15 ± 362.37	0.068
% CD86	24.11 ± 13.78	1494.07 ± 4445.82	0.649
MIF 86	639.67 ± 37.13	574.51 ± 204.14	0.466
% CD163	82.39 ± 9.20	153.51 ± 217.85	0.671
MIF 163	**3010.81 ± 2422.58**	**1698.99 ± 793.94**	**0.013 ****
% CD206	8.42 ± 5.07	118.12 ± 333.30	0.605
MIF 206	**510.91 ± 54.65**	**425.07 ± 197.91**	**0.000 *****
% 11c/86	23.16 ± 12.93	189.30 ± 510.08	0.605
% 163/206	**5.42 ± 3.46**	**4.24 ± 7.72**	**0.027 ***
Classical monocytes			
%	89.68 ± 4.39	92.26 ± 2.55	0.092
% CD11c	**91.57 ± 9.69**	**97.88 ± 2.13**	**0.005 ****
MIF 11c	**709.87 ± 157.26**	**928.22 ± 249.20**	**0.008 ****
% CD86	21.13 ± 13.76	22.16 ± 14.10	0.779
MIF 86	586.91 ± 15.35	598.80 ± 27.90	0.291
% CD163	89.06 ± 9.86	86.85 ± 9.09	0.269
MIF 163	**3053.45 ± 2405.70**	**1905.96 ± 555.69**	**0.041 ***
% CD206	6.79 ± 4.50	6.18 ± 7.92	0.166
MIF 206	**478.14 ± 39.38**	**469.28 ± 129.75**	**0.016 ****
% 11c/86	20.14 ± 12.67	22.05 ± 14.04	0.741
% 163/206	6.64 ± 4.19	5.98 ± 7.64	0.137
Intermediate			
%	3.77 ± 2.37	2.77 ± 1.27	0.290
% CD11c	**97.99 ± 2.65**	**99.66 ± 0.39**	**0.004 ****
MIF 11c	**1518.99 ± 474.10**	**1976.88 ± 536.37**	**0.013 ***
% CD86	66.96 ± 26.87	70.89 ± 17.14	0.974
MIF 86	832.39 ± 98.82	871.68 ± 104.54	0.488
% CD163	80.41 ± 15.61	79.65 ± 13.11	0.644
MIF 163	3678.20 ± 5385.99	1849.45 ± 629.27	0.488
% CD206	36.37 ± 16.27	33.21 ± 15.31	0.668
MIF 206	**575.94 ± 124.37**	**517.74 ± 247.86**	**0.041 ***
% 11c/86	66.79 ± 26.82	70.88 ± 17.13	0.974
% 163/206	34.37 ± 16.14	30.24 ± 15.10	0.530

Table 3. *Cont.*

	Nociceptive (n = 18)	Mixed (n = 20)	
	M ± SD	M ± SD	*p*
Non-classical			
%	**3.72 ± 2.69**	**1.82 ± 1.09**	**0.037 ***
% CD11c	74.93 ± 30.12	87.43 ± 18.27	0.409
MIF 11c	**1522.18 ± 466.66**	**1999.55 ± 447.14**	**0.005 ****
% CD86	60.33 ± 31.01	73.32 ± 17.57	0.276
MIF 86	828.17 ± 104.68	864.42 ± 98.72	0.269
% CD163	13.68 ± 7.98	12.84 ± 7.77	0.520
MIF 163	941.04 ± 357.84	775.93 ± 89.13	0.276
% CD206	12.46 ± 10.10	10.40 ± 7.58	0.869
MIF 206	729.17 ± 465.05	443.38 ± 70.24	0.060
% 11c/86	60.24 ± 31.02	73.22 ± 17.65	0.283
% 163/20	4.45 ± 4.52	3.54 ± 5.76	0.209

M—mean; SD—standard deviation; * $p \leq 0.05$ ** $p \leq 0.01$ *** $p \leq 0.001$. %—percentage; MFI—mean fluorescence intensity; *p*: significance. The statistically significant differences are highlighted in bold.

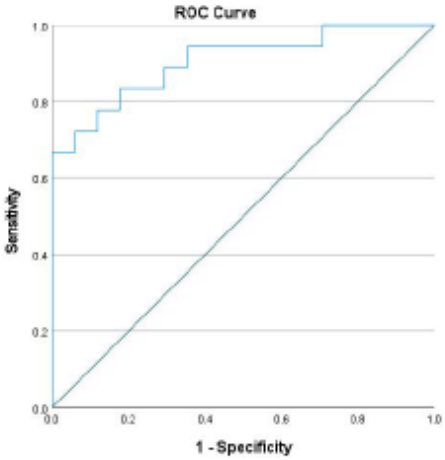

Figure 2. ROC curve of CD11c, CD163, and CD206 as biomarkers of nociceptive pain.

2.4. *Analgesic Drugs and Monocyte Biomarkers*

We also analyzed if the treatment with opioids and paracetamol interfered with the monocyte biomarkers. Consequently, we conducted a comparison of monocyte biomarker levels between patients who were receiving treatment with opioids and paracetamol and those who were not. Our findings indicated that CD163 was the sole biomarker with statistical significance. In patients receiving opioid therapy, the percentage of monocytes expressing CD163 was lower than those not receiving opioids (values of total monocytes and the three subtypes), as shown in Table 4. These levels are higher in patients receiving paracetamol treatment, particularly in non-classical monocytes. No significant differences were found between the studied monocyte biomarkers and the equivalent dose of morphine.

Table 4. Differences between CD163 values in patients receiving opioid therapy and in patients without opioids.

		Without Opioids	Opioid Therapy	
		M ± SD	M ± SD	*p*
Monocytes	%163	126.57 ± 169.27	80.54 ± 9.92	0.135
	MIF163	2645.37 ± 2041.58	2246.61 ± 985.25	0.441
Classical monocytes				
	%163	91.1 ± 8.1	86.6 ± 10.7	0.108
	MIF163	2858.3 ± 1978.5	2303.3 ± 1064.9	0.279
Intermediate monocytes				
	%163	85.7 ± 9.2	74.0 ± 16.3	0.010 *
	MIF163	3363.0 ± 4288.1	1755.8 ± 440.3	0.051
Non-classical monocytes				
	%163	13.9 ± 10.1	11.4 ± 5.1	0.334
	MIF163	936.97 ± 362.22	762.52 ± 128.76	0.056

M—mean; SD—standard deviation; * $p \leq 0.05$. %—percentage; MFI—mean fluorescence intensity; *p*: significance.

2.5. *Monocyte Characterization and Dementia*

In order to correlate the phenotypic markers in monocytes with dementia, we analyzed the levels of different monocyte subsets and CD in patients with and without dementia of different subtypes.

As shown in Table 5, we found some significant differences between the biomarkers and dementia. One finding is that patients with dementia have a higher relative concentration of monocytes than those without dementia (11.08% vs. 4.73%, $p = 0.037$). Notably, patients with vascular dementia exhibited the lowest levels of relative concentrations of monocytes.

Table 5. Monocyte biomarkers and dementia.

	Without Dementia	With Dementia	
	M ± SD	M ± SD	*p*
Monocytes			
%	**4.73 ± 2.11**	**11.08 22.11**	**0.037 ***
% CD11c	88.29 ± 10.05	330.11 ± 996.73	0.125
MIF 11c	784.79 ± 256.61	835.32 ± 321.77	0.305
% CD86	29.65 ± 14.25	821.8 ± 3319.12	0.271
MIF 86	631.93± 24.31	605.24 ± 154.41	0.791
% CD163	85.39 ± 7.11	120.54 ± 162.75	0.335
MIF 163	**3420.37 ± 2557.46**	**2108.14 ± 1055.71**	**0.032 ***
% CD206	12.68 ± 9.22	67.72 ± 248.83	0.138
MIF 206	**562.85± 129.08**	**445.58 ± 123**	**0.001 ****
% 11c/86	28.83 ± 13.73	112.91 ± 380.71	0.236
% 163/206	**9.2 ± 8.5**	**4.1 ± 3.2**	**0.010 ****

Table 5. *Cont.*

	Without Dementia	With Dementia	
	M ± SD	**M ± SD**	***p***
Classical monocytes			
%	87.8 ± 5.6	91 ± 4.4	0.054
% CD11c	91.1 ± 10.3	94.3 ± 8.6	0.097
MIF 11c	726 ± 234.2	847.4 ± 272.2	0.081
% CD86	27.5 ± 13.9	21.4 ± 13.4	0.114
MIF 86	591.7 ± 11.9	593.5 ± 25.7	0.991
% CD163	**93.6 ± 8.2**	**87.5 ± 9.3**	**0.012 ***
MIF 163	3497.5 ± 2534.9	2265 ± 978.4	0.063
% CD206	10.6 ± 9.2	6.1 ± 4.5	0.087
MIF 206	**528.2 ± 127**	**456.1 ± 40.6**	**0.002 ****
% 11c/86	26.5 ± 13.3	20.6 ± 12.8	0.114
% 163/206	10.4 ± 9	6 ± 4.3	0.089
Intermediate			
%	**4.7 ± 2.5**	**3.1 ± 2.1**	**0.028 ***
% CD11c	98.6 ± 2.1	98.4 ± 3.2	0.832
MIF 11c	1653.2 ± 565.9	1791.9 ± 552.9	0.417
% CD86	71.5 ± 20.5	69.7 ± 21.4	0.920
MIF 86	820.3 ± 98.1	857.6 ± 101.8	0.158
% CD163	**87.4 ± 10**	**78.5 ± 14.1**	**0.021 ***
MIF 163	3726.8 ± 5665.9	2320.9 ± 1730.1	0.085
% CD206	42.5 ± 15.9	34.8 ± 15.8	0.213
MIF 206	607.6 ± 226.6	518.5 ± 130.7	0.107
% 11c/86	71.4 ± 20.4	69.6 ± 21.4	0.903
% 163/206	40.5 ± 15.9	32.1 ± 15.6	0.095
Non-classical			
%	4.27 ± 3.41	2.87 ± 2.46	0.221
% CD11c	76.5 ± 30	79.5 ± 25.5	0.656
MIF 11c	1636.6 ± 577.9	1721.2 ± 554.9	0.601
% CD86	61.5 ± 27.9	64 ± 26.3	0.648
MIF 86	828.5 ± 94.4	848.7 ± 127.4	0.664
% CD163	13.3 ± 9.7	12.9 ± 8.2	0.764
MIF 163	870.87 ± 339.24	871.86 ± 296.75	0.714
% CD206	14.6 ± 13.8	11.5 ± 9.3	0.368
MIF 206	**752.9 ± 447.3**	**507.9 ± 218.5**	**0.008 ****
% 11c/86	61.4 ± 27.9	63.9 ± 26.3	0.640
% 163/206	3.8 ± 4	3.9 ± 5.2	0.490

M—mean; SD—standard deviation; * $p \leq 0.05$ ** $p \leq 0.01$ *** $p \leq 0.001$. MFI—mean fluoresce intensity; *p*: significance. The statistically significant differences are highlighted in bold.

Moreover, in the monocytes of dementia patients, the expression levels of CD163, CD206, and the ratio of CD163/CD206 are lower than those detected in patients without dementia (CD163—3420.37 vs. 2108.14 MFI, $p = 0.032$; CD206—562.85 vs. 445.58 MFI,

$p = 0.001$; CD163/CD206—9.2 vs. 4.1, $p = 0.01$). Moreover, the classic and intermediate monocytes of patients with dementia show a significant decrease in the percentage of monocytes expressing CD163, while the decrease in the expression levels of CD206 is mainly observed in classic and non-classic monocytes, when compared with patients without dementia (classic—528.2 vs. 456.2 MFI, $p = 0.002$; non-classic—752.9 vs. 447.3 MFI, $p = 0.008$) (Table 5).

When comparing biomarkers with types of dementia, there are statistically significant differences, particularly in the relative concentration of monocytes, as previously mentioned, and in the expression levels of CD206, which are highest in patients with vascular dementia (17.06% vs. 4.73%, $p = 0.028$ and 465.70 vs. MFI 419.85, $p = 0.007$, respectively).

When we analyzed the different subsets of monocytes in patients with different types of dementia, in intermediate monocytes, we did not observe significant differences among Alzheimer's disease, vascular, and other types of dementia. Conversely, we observed a significant increase ($p = 0.045$) in the expression of CD206 in non-classical monocytes among patients with vascular dementia (MFI 561.1), whereas the lowest expression was observed in patients with Alzheimer's disease (MFI 482.0). Our findings showed a significant decrease ($p = 0.049$) in the expression levels of CD86 (MFI 579.3) in classical monocytes of Alzheimer's disease patients compared to those with other dementias (MFI 597.1). Moreover, the relative percentage of CD163 was significantly higher in Alzheimer's patients than in those patients with vascular dementia (MFI 93.6 vs. MFI 84.9, $p = 0.036$).

2.6. Monocyte Characterization and Opioids and Other Analgesics Used to Control Pain in These Patients

When we compared the percentages of monocytes, and of the monocyte subsets characterized by the different CD, referred to before in patients receiving opioids and in patients that did not receive opioids, we did not find statistically significant differences between the two groups of patients. We had identical findings when comparing patients receiving other analgesics, such as paracetamol and/or NSAIDs, with patients without any pain treatment.

3. Material and Methods

For this study, we obtained clinical and individual data and blood samples from 53 palliative patients with non-oncological diseases, followed by a palliative care team between 1 September and 31 December 2021.

This study is an observational, analytical, cross-sectional, non-interventional investigation that utilizes the medical and nursing records of patients with chronic pain.

The North Regional Health Administration of Portugal (ARS Norte) and the Faculty of Medicine (FMUP) Ethics Committee accepted the research procedures. The research was conducted following the principles of the Declaration of Helsinki. In compliance with ethical guidelines on confidentiality, data anonymity, and voluntary withdrawal, the participants or their representatives provided informed consent before study enrollment.

We collected individual and clinical data by reviewing the records in the patient's clinical files, which were then saved in a secure Excel sheet [23]. Each patient was assigned an alphanumeric code to ensure patient confidentiality, known only to the researcher. Following the European General Data Protection Regulation (GDPR), the Excel files will be eliminated upon the completion of the study and publication of the results. The data included age, gender, pain type and intensity, opioid and other analgesic drugs, such as nonsteroidal anti-inflammatory drugs (NSAIDs) and acetaminophen, and their corresponding doses. Additionally, we recorded whether the patient's pain was controlled during blood sample collection.

We also obtained information regarding the diagnosis and type of dementia. In cases where patients had severe dementia, we utilized the Pain Assessment in Advanced Dementia Scale (PAINAD) [22] to differentiate those who may present uncontrolled pain. Based on the PAINAD scores, we categorized patients into three groups, scores below 5,

scores between 5 and 7, and scores between 8 and 10, to investigate the potential correlation between these scores and mild, moderate, or severe pain, respectively. We administered the numeric pain scale for patients who could self-report their pain (Rodriguez, 2001).

To analyze the expression of monocyte biomarkers, we selected CD11c [24], CD86 [25], CD163 [26] and CD206 [27] both absolute and relative concentration. We conducted a receiver–operator characteristic (ROC) curve analysis to determine the area's significance under the curve and establish a cut-off point for the relevant markers.

3.1. Evaluation of Monocytes Subsets by Flow Cytometry

We collected peripheral blood samples in EDTA tubes to analyze the monocytes. Monocytes were classified into three subsets based on the expression levels of CD14 and CD16. These subsets are the classical monocytes (CD14+CD16−), non-classical monocytes (CD14−CD16+), and intermediate monocytes (CD14+CD16+) [11]. Following a 15 min incubation in the dark at room temperature, erythrocytes were lysed using a BD. Pharm Lyse™ reagent according to the manufacturer's protocol (BD Pharmingen, BD Biosystems, San Diego, CA, USA). Cells were run through a FACS Canto II flow cytometer (BD Biosystems), and at least 100,000 events were collected using FACS DIVA software (BD Biosystems). Data were studied with the Kaluza Software (Beckman Coulter, Jersey City, New Jersey), and the results are expressed using a total of monocytes (CD14+) and the percentage of each monocyte subset based on the cells' positivity for CD14 and CD16 expression [monocytes classic (CD14+/CD16−), non-classic (CD14+/CD16++) and intermediates (CD14++/CD16+)].

3.2. Evaluation of Monocyte Subsets and Membrane Proteins Using Flow Cytometry

The monocyte analysis was performed in peripheral blood samples collected in EDTA tubes to identify the monocyte subsets and characterize the transmembrane protein receptors related to its recognized functions.

We stained 200 µL of whole blood with the following monoclonal antibodies (mAbs): anti-CD14 BD Pharmingen™ (APC), anti-CD16 BD Horizon™ (BV500) mAbs; anti-CD11c BD Pharmingen™ (FITC), anti-CD163 BD Pharmingen™ (PE), anti-CD86 BD Pharmingen™ (PerCP-Cy5.5), and anti-CD206 BD Horizon™ (BV421). Following a 15 min incubation in the dark at room temperature, erythrocytes were lysed using a BD. Pharm Lyse™ reagent according to the manufacturer's protocol. Cells were run through a FACS Canto II flow cytometer (BD Biosystems), and at least 100,000 events were collected using FACS DIVA software (BD Biosystems). Data were studied with the Kaluza Software (Beckman Coulter), and the results were expressed in a percentage of positive cells for each marker and respective mean fluorescence intensity (MFI). Each marker was analyzed in the total monocyte population (CD14+). Additionally, a sub-analysis was performed based on monocyte classification, using CD14 and CD16 expression, into classical ($CD14^{+}CD16^{-}$), non-classical ($CD14^{-}CD16^{+}$), and intermediate ($CD14^{+}CD16^{+}$) monocytes. In each monocyte subset, the expressions of CD11c, CD163, and anti-CD206 were determined.

3.3. Statistical Analysis

We used SPSS (version 28.0 for Windows, IBM Corp., Armonk, NY, USA) for statistical analysis. Descriptive statistics measures such as absolute and relative frequencies, means, and standard deviations were employed for data analysis. Additionally, inferential statistics were also utilized. We set the level of significance (α) at ≤ 0.05 to reject the null hypothesis. The Student's *t*-test for independent samples, one-way ANOVA, Mann–Whitney U test, Kruskal–Wallis test, and logistic, binary, and multinomial regressions were used. The normality of distribution was analyzed with the Kolmogorov–Smirnov test. The homogeneity of variances was evaluated with Levene's test. Multicollinearity was analyzed with VIF and Tolerance. ROC analysis and the calculation of the cut-off value for the biomarkers, as well as the specificity, sensitivity, positive predictive, and negative predictive values, were performed.

We incorporated several variables into the analysis, including sex, age, type and intensity of pain, opioid type and dosage, and other analgesics like nonsteroidal anti-inflammatory drugs (NSAIDs) and paracetamol, as well as absolute (MFI) and relative concentration levels of CD11c, CD86, CD163, and CD206. In our analysis, we factored in the type of dementia, since it was prevalent among most patients, and controlled pain time for patients presenting with chronic pain but whose pain was not present during blood collection. In cases where patients had severe dementia, we relied on the Pain Assessment in Advanced Dementia Scale (PAINAD) [22] to identify pain that was not controlled. We included additional variables that might have an association with our results, including the body mass index (BMI), measures of renal function applying the Cockcroft–Gault formula [28], and functionality using the Karnovsky scale [29]. The Mini-Nutritional Assessment scale evaluated the nutritional status [30].

Due to a lack of evidence in published clinical data regarding this issue, it is not possible to firmly assume the size of the changes [31]. We assumed a grouping of two with a 1:1 allocation, an effect size d of 0.8, and a significance alpha of 0.05. With 20 participants in each arm, we had 80% power to detect minimal differences. In addition, the authors expected to lose another 20% of the participants during the follow-up. Therefore, we used a larger sample size to promote a more statistically rigorous method.

3.4. Inclusion Criteria

Our study involved patients followed by a specialized palliative care team in the northern region of Portugal with non-oncological illnesses. As previously stated, we only included patients who provided informed consent directly or through their legal representatives.

4. Discussion and Conclusions

It is difficult to assess pain in patients receiving palliative or end-of-life care or those with severe dementia, cognitive impairment, or speech disorders, as we cannot solely rely on self-reporting [3,4].

Physical symptoms such as pain, fatigue, and sleep disturbances and psychosocial factors such as stress, coping style, and the availability of emotional support interact in a bidirectional manner and are present at varying levels. These dynamics create a highly personalized disease experience that calls for an individualized, multimodal therapeutic approach [32,33]. Consequently, there is an urgent need to explore non-invasive pain biomarkers that can aid in characterizing pain and customize therapeutic strategies individually.

Several preclinical and clinical studies have investigated the hypothesis that biomarkers can be utilized to identify and quantify pain. A preclinical study shows that inflammatory and neuropathic pain have different biomarkers [34]. Further investigations provided mixed results. For instance, cystatin C levels in cerebrospinal fluid appear to be a predictive marker for postherpetic neuralgia in patients with varicella-zoster virus and a pain marker in women experiencing labor pain. Nevertheless, these biomarkers have no correlation with pain duration or intensity. Studies examining potential biomarkers for chest pain have revealed that cardiac markers utilized for diagnosing cardiac disease and prognosis are associated with tissue damage rather than pain [34].

Previously, many routine peripheral blood parameters have been used as peripheral novel diagnostic, prognostic, and predictive response markers in several diseases [7–9,35,36], including dementia [6]. We have recently demonstrated that membrane platelet proteins have some value as pain biomarkers, namely for pain subtype classification and pain intensity characterization [8].

This study assesses whether monocytes, their subtypes, and membrane proteins, such as CD11c, CD86, CD163, and CD206, could serve as non-invasive peripheral biomarkers for identifying and characterizing pain in patients with severe dementia. Our findings suggest that the relative concentrations of monocytes, specifically the percentage of classic monocytes, may serve as valuable biomarkers for pain, irrespective of sex, the presence of dementia, and the type of pain. Moreover, the most evident alterations were found in

the levels of CD11c, CD163, and CD206 in classic monocytes, which may contribute to characterizing the pain subtype. We did not detect any changes in CD86 expression related to pain and dementia.

CD11c is a complement receptor often exploited as a single marker to track murine dendritic cells [24]. CD86 is a constitutively expressed phenotypic marker on interdigitating dendritic cells (DCs), Langerhans cells, peripheral blood DCs, memory and germinal center B cells, and macrophages [25]. CD163 is a scavenger receptor for haptoglobin-hemoglobin complexes expressed mainly by monocytes and macrophages induced by inflammatory stimuli [26]. Finally, CD206 is a mannose receptor primarily found on the surface of alternatively activated macrophages and serves as a pattern recognition receptor, contributing to innate and adaptive immunity [27].

This study demonstrated that individuals experiencing pain had higher levels of CD206 and classical monocytes and a lower CD163/CD206 ratio, suggesting the potential use of these parameters as biomarkers for pain. They are independent of gender, dementia, and pain treatment. In fact, in patients with pain, we found a significant increase in the percentage of classic monocytes and the percentage of these cells expressing CD206 and a decrease in the ratio of CD163/CD206 compared to patients without pain. None of these parameters varied with sex, dementia, or treatment with paracetamol and opioids. If these findings are validated in future studies involving a larger number of patients, they should be incorporated into the pain identification process in the peripheral blood of palliative patients with dementia. For patients with well-controlled pain, in comparison with those with non-controlled pain, we did not find statistically significant differences, including in patients with well-controlled pain for seven or more days. These data are relevant because we could expect a reduction in monocyte activation with pain control [13,35,37]. However, it may mean that some underlying mechanisms and causes of pain may be equally active, and that there is only pain desensitization.

Despite the limited sample size, which precluded the assessment of pain types other than nociceptive and mixed pain, we could identify monocyte biomarkers associated with certain types of pain. The levels of CD11c are significantly higher in patients with mixed pain, while the expression levels of CD163 and CD206 are significantly higher in patients with nociceptive pain, with the latter being independent of dementia, as these patients have lower levels of these markers.

Another interesting finding is that the percentage of CD163 in intermediate monocytes showed less expression in patients receiving opioid treatment. Freshly isolated peripheral blood monocytes express a relatively low level of CD163, but this expression increases with the differentiation of such cells into macrophages [26], a role assigned mainly to intermediate monocytes [11,36]. Our results confirm that opioids may substantially affect monocytes' inactivation, as shown by others [38,39], confirming their anti-inflammatory effect. On the other hand, in patients receiving paracetamol, only an increase in the ratio of CD163/CD206 was detected in non-classic monocytes. According to the literature [40–42], there are only references to the increase/activation of CD163 in monocytes frequently related to the liver injury induced by paracetamol. However, we did not find liver disease or dysfunction in these patients.

Monocytes can be used as predictors of dementia, as the relative concentration of total monocytes is higher in patients with dementia, particularly in Alzheimer's disease and vascular dementia compared with non-demented controls. These findings are in agreement with others [43,44], and the hyperactivation of monocytes has been described as a potential contributor to the progression from mild cognitive impairment to Alzheimer's disease [45]. While the implication of microglia is well recognized, the exact contribution of peripheral monocytes or macrophages is still largely unknown, especially concerning their role in the various stages of Alzheimer's disease [45].

Additionally, we did not observe any differences in the levels of monocyte subtypes, contrasting with the findings reported by other authors [45]. However, it should be noted

that this report included healthy individuals as controls, in contrast to our study which had only palliative patients, and the controls were those without pain.

Furthermore, the decreased expression levels of CD86 and CD206 could be potential markers of Alzheimer's disease and vascular dementia, respectively [45]. Conversely, elevated levels of CD163 and CD206 were observed in patients without dementia, suggesting that these markers may serve as negative predictors of dementia. These findings are not in agreement with the literature [45–51], but they seem to be related with other conditions or are related with the risk of Alzheimer's disease development, which was not the scope of our study.

Taken together, these findings suggest that the levels of specific monocyte subtypes, namely the classic subtype, along with their associated CD11c, CD163, and CD206 phenotypes may hold value as objective pain biomarkers for palliative patients with dementia. This is a significant advantage as these biomarkers can be quantified without requiring active patient involvement.

The main limitation of our cross-sectional study is the small sample size, which limits the formation of even groups. In this sense, a confirmatory study in a larger cohort of patients should be performed in order to confirm our findings. Additionally, the fact that the pain observed in severe dementia patients can result from different causes and mechanisms, and the fact that patients with pain do not respond similarly to the same treatment may also increase data dispersion. Also, a subtype of dementia may be partially linked to a specific mechanism of neuroinflammation, and therefore this could influence the results [43–45,52,53]. Finally, some confounding factors, such as evolution in time and previous treatments, that could affect pain assessment in dementia pain were not explored in this study.

To our knowledge, this is one of the most extensive pain biomarker studies conducted to date and the only study to compare patients with non-oncological pain using specific monocyte biomarkers. Nevertheless, given the small sample size, further studies are warranted to confirm the viability of these markers as indicators of pain and dementia.

For vulnerable and dependent patients who cannot provide a self-report of symptoms, identifying pain biomarkers, such as the ones presented in this study, can aid in adjusting therapeutic strategies in line with the WHO ladder [2], contributing to a faster and more adequate control of pain, and a reduction in associated suffering.

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DISCUSSÃO E CONCLUSÕES

Neste trabalho, foi efectuada uma extensa revisão da literatura e reflexão sobre o papel das características individuais na abordagem farmacológica do doente com dor, assim como foram identificados potenciais alvos terapêuticos, tendo por base o objectivo de contribuir para uma medicina de precisão, desde o diagnóstico à abordagem farmacológica.

Nos estudos laboratoriais, foi possível definir um conjunto de biomarcadores plaquetários e monocitários que poderão ter um papel relevante na identificação e caracterização da dor, sobretudo nos doentes sem capacidade de autorrelato, como acontece nos doentes com demência, permitindo uma abordagem terapêutica dirigida e mais eficiente.

ABORDAGEM FARMACOLÓGICA EM MEDICINA DA DOR – PRINCIPAIS CONTRIBUTOS

A abordagem médica ao doente com dor pressupõe uma anamnese cuidada, com uma história clínica e um exame físico centralizado nas queixas do doente (Busse et al., 2017; Direção-Geral da Saúde, 2010; Hegmann et al., 2014; Scottish Intercollegiate Guidelines Network, 2013; Tauben, 2013; Treede et al., 2019). Assume-se como objetivo principal o alívio ou mesmo o controlo da dor, tendo por base a escada analgésica da OMS (Anekar & Cascella, 2023), focada nos mecanismos de ação e na eficácia das terapêuticas farmacológicas para a dor.

Das várias *guidelines* e recomendações nacionais e internacionais consultadas, há um foco na evidência do uso de determinado grupo farmacológico na presença de uma doença, condição ou tipo de dor (Busse et al., 2017; Direção-Geral da Saúde, 2010; Dowell et al., 2016; M. Li et al., 2020; Pedro & Silva, 2017; Raffaeli et al., 2021; Raja et al., 2020; Scottish Intercollegiate Guidelines Network, 2013; Tauben, 2013; Treede et al., 2019).

Por exemplo, na dor neuropática está aconselhada a utilização em primeira linha de gabapentinóides e de antidepressivos (Colloca et al., 2017; Finnerup et al., 2021; Jensen et al., 2011). Mas, as orientações de abordagem clínica não consideram as

características individuais dos doentes nem da própria dor neuropática, que nem sempre tem características neuroimunes idênticas.

No caso da dor moderada a intensa, as recomendações atuais incentivam o uso de opióides (Anekar & Cascella, 2023; Busse et al., 2017; Direção-Geral da Saúde, 2010; Dowell et al., 2016; Pedro & Silva, 2017). No entanto, mais uma vez, não se consideram as características individuais dos doentes para ajuste das doses e posologias, não tendo em conta as diferenças farmacocinéticas entre os vários opióides aprovados para o tratamento da dor. Há ainda um grande desconhecimento acerca da farmacogenómica e em como estas características individuais poderão influenciar a prescrição médica no futuro próximo (Cornett et al., 2020).

As características corporais e a funcionalidade hepática e renal são essenciais para a farmacocinética dos fármacos mais prescritos para o tratamento da dor (paracetamol, AINEs e opióides). Por estas razões, a caracterização individual deve ser central na escolha do fármaco mais eficaz e seguro, e ainda na dose e posologia mais adequadas (Ribeiro et al., 2017, 2021, 2022, 2023).

No caso do paracetamol, este pode ser utilizado na dor aguda e na dor crónica e em todos os tipos de dor, assim como em todos os graus de intensidade de dor. Apresenta um perfil de segurança confortável, e possuiu um mecanismo de ação, ainda não totalmente esclarecido (Alchin et al., 2022). Tem como grande vantagem ser um fármaco multimodal e sinérgico com todos os restantes fármacos, particularmente com os opióides e com os AINEs (Alchin et al., 2022; Bannwarth & Pehourcq, 2003; Bertolini et al., 2006; Forrest et al., 1982; Mian et al., 2018; Yin et al., 2022). Os ajustes de dose são fundamentais atendendo às características individuais; sendo um medicamento hidrossolúvel, em doentes idosos e doentes frágeis, que têm menos água corporal, a dose deverá ser consideravelmente reduzida (para 500mg a 650mg em cada administração), atendendo a que se atinge a concentração máxima de distribuição do fármaco com doses mais baixas. Por outro lado, deverá manter-se a posologia máxima de 6 em 6 horas. O paracetamol é ainda um fármaco com baixa probabilidade de interação com outros fármacos, atendendo ao seu metabolismo de fase 2 de biotransformação hepática (Alchin et al., 2022; Bannwarth & Pehourcq, 2003; Forrest et al., 1982; Mian et al., 2018).

No caso dos AINEs, este grupo de fármacos demonstra ter igual potencial de eficácia analgésica (Ribeiro et al., 2022). Salienta-se que o naproxeno demonstra ter um perfil de segurança cardiovascular inferior aos coxibes e a outros inibidores não seletivos da COX (Bally et al., 2017; Nissen et al., 2016; Ribeiro et al., 2022), com um nível de evidência A, o que contrasta com algumas das *guidelines* internacionais sobre o uso de AINEs, incluindo a portuguesa (Direção Geral da Saúde, 2013). Os presentes resultados levam a propor que os AINEs hidrossolúveis, como o diclofenac, o etodolac e os coxibes, tenham uma redução de dose nos doentes idosos e nos doentes frágeis, que apresentam menor quantidade de água corporal, e para os AINEs lipossolúveis deverá ocorrer um ajuste da posologia nos doentes idosos e obesos, nomeadamente com o alargamento temporal das administrações (força de recomendação B). Os AINEs com melhor perfil de segurança hepático, para doentes alcoólicos, polimedicados e idosos, serão a acemetacina e o diclofenac (força de recomendação C), pois são os que apresentam metabolismo de fase 2 de biotransformação, que no caso da acemetacina é totalmente de fase 2. Relativamente ao perfil de segurança cardiovascular e renal, o AINE mais seguro será a acemetacina (força de recomendação B), pois apresenta uma eliminação renal muito inferior aos restantes (apenas cerca de 40% do fármaco é eliminado pelo sistema urinário), sendo que o presente estudo propõe que a acemetacina seja o fármaco de eleição em doentes com taxa de filtração glomerular (TFG) entre 45ml/min e 60ml/min. Nos doentes com TFG inferiores a 45ml/min, a alternativa para um fármaco anti-inflamatório de ser um corticóide (Fernandez-Juarez et al., 2018; Mose et al., 2021; Nakahama & Sakaguchi, 2001; Qin et al., 2020).

Relativamente à dor com características inflamatórias, os AINEs são geralmente aconselhados, em períodos curtos de tratamento (Crofford, 2013; M. Li et al., 2020; Pelletier et al., 2016), que não deverão ultrapassar 10 dias (Aminoshariae et al., 2016; Schjerning Olsen et al., 2011).

O tratamento agressivo da dor proposto pela generalidade das sociedades científicas e peritos da área (Busse et al., 2017; Hegmann et al., 2014; Pedro & Silva, 2017; Ribeiro et al., 2021; Scottish Intercollegiate Guidelines Network, 2013; Tauben, 2013) assim como o tratamento de outros sintomas associados à doença ou a efeitos secundários dos fármacos deverá ter em conta as limitações funcionais do doente e a sua expectativa de

vida. Com efeito, alguns destes sintomas, como a sonolência e a obstipação, poderão ter um efeito protetor da sobrecarga do cuidador e não prejudicam o bem-estar de doentes em fim de vida (Ribeiro et al., 2023). A abordagem individualizada é fundamental, com a respectiva ponderação entre os riscos e os benefícios de qualquer intervenção, mas sempre focada no bem-estar e no conforto do doente.

De acordo com os resultados encontrados no âmbito da investigação desta tese, é fundamental escolher o opióide mais adequado a ser utilizado perante um doente, que apresenta uma determinada condição clínica, e que tem características farmacocinéticas individuais. Será extremamente relevante, sobretudo para doentes em cuidados paliativos, adaptar a dose, a posologia e a titulação do opióide às características farmacocinéticas do doente (Ribeiro et al., 2021; Ribeiro et al., 2023).

Das características farmacodinâmicas mais relevantes, salientamos que, na dor crónica, o tapentadol e o tramadol poderão apresentar melhor perfil de eficácia, pelos seus mecanismos de ação. De facto, o tapentadol é um opióide *major* que também tem atividade noradrenérgica e o tramadol é um opióide *minor* que também tem actividade serotoninérgica e noradrenérgica (Barakat, 2019; Beakley et al., 2015; Grond & Sablotzki, 2004; Tzschentke et al., 2014).

Relativamente ao perfil de segurança, relacionado com as características farmacocinéticas dos medicamentos, destacamos a identificação da morfina e da hidromorfona como únicos opióides hidrossolúveis, comercializados em Portugal e aprovados para o tratamento da dor em ambulatório (Franken et al., 2016; Hoskin & Hanks, 1990). Em 2023, a hidromorfona foi descontinuada em Portugal por opção do laboratório farmacêutico que detém a sua patente, o que tornou a morfina o único opióide hidrossolúvel em Portugal.

Tendo em conta as características individuais do doente, no caso de doentes frágeis e idosos (com maior probabilidade de desidratação e com menor quantidade de água corporal), o volume de distribuição da morfina e da hidromorfona é inferior, o que significa que as titulações devem ser realizadas recorrendo a aumentos de dose mais baixos em relação à dose equivalente de morfina anterior. Por outro lado, o tempo de semivida tem tendência a diminuir, pois a quantidade de fármaco em distribuição atinge

mais facilmente o pico de concentração plasmática (Franken et al., 2016; Hoskin & Hanks, 1990; Klimas & Mikus, 2014; Ribeiro et al., 2021). No caso inverso, em opióides lipossolúveis, nos doentes obesos e idosos observamos uma tendência para o prolongamento da sua ação, pelo que a posologia deve ser alargada e as titulações mais espaçadas no tempo, limitando a probabilidade de sobreposição de doses e a consequente sobredosagem (Ashburn et al., 2003; Bista et al., 2015; Dowell et al., 2016; Ribeiro et al., 2021; Veríssimo, 2014). Esta realidade é ainda mais acentuada nos doentes idosos, pois no envelhecimento assistimos a um aumento da proteína plasmática α1-glicoproteína ácida, que apresenta afinidade ligeira a moderada para os opióides, aumentando o seu tempo de semivida plasmático (Katzung et al., 2014; Ribeiro et al., 2017, 2021).

Relativamente à metabolização hepática, apenas a morfina e o tapentadol apresentam metabolismo de biotransformação de fase 2 (Franken et al., 2016; Göhler et al., 2013; Hoskin & Hanks, 1990; Terlinden et al., 2007; Xu et al., 2010). Nesse contexto, são os fármacos de eleição para doentes polimedicados, idosos e doentes com abuso crónico do álcool, pelo menor risco de interações farmacológicas e prolongamento da metabolização e consequentes efeitos adversos provocados.

Quanto à eliminação renal, a buprenorfina é o opióide com excreção biliar praticamente total, sendo o fentanilo um opióide com grande excreção renal. No entanto, como este último é eliminado sobre a forma de metabolitos inativos, se ocorrer um atraso na sua eliminação, o fentanilo não apresenta probabilidade de provocar sobredosagem nem os respetivos efeitos adversos (Ashburn et al., 2003; Bista et al., 2015; Molfenter et al., 2019; Shi et al., 2019; Van Zee & Fiellin, 2019). Assim sendo, nos doentes idosos e nos doentes com insuficiência renal, o fentanilo e a buprenorfina poderão apresentar melhor perfil de segurança pela reduzida alteração à sua farmacocinética decorrente destas condições.

BIOMARCADORES DA DOR – O PAPEL DAS PLAQUETAS

Várias alterações plaquetárias estão associadas a patologias que cursam frequentemente com dor (Cao et al., 1998; Flaumenhaft, 2004; Kwon et al., 2020; Salem, 1980). Graham e colegas demonstraram, em 2004, que as plaquetas aumentam a expressão de receptores membranares de taquicininas em resposta à libertação da substância P e outras taquicininas, como as neurocininas A e B, as endocininas A, B, C e D, as hemocininas e outras menos expressivas (Graham et al., 2004).

Em doentes com dor, em resposta à substância P, há um aumento significativo da agregação plaquetária, estimulada pela ativação de receptores membranares da substância P e da neurocinina B, tais como o NK1 e o NK3, respectivamente (Flaumenhaft, 2004).

Vários marcadores membranares das plaquetas ainda não estudados poderão estar igualmente associados à dor. Caso seja comprovada esta ligação, será conseguido o reforço e eventualmente a padronização de um conjunto de marcadores celulares e extracelulares relacionados com a dor, permitindo uma identificação e caracterização precisa desta condição, permitindo uma abordagem terapêutica mais dirigida.

Nestes trabalhos, foram seleccionados vários potenciais biomarcadores plaquetários, envolvidos na identificação, adesão e agregação plaquetária, e para os quais existem estudos em que a sua expressão se altera na presença de patologias que frequentemente apresentam dor (Barbar et al., 2020; Daviet & McGregor, 1997; Dostert et al., 2019; Heemskerk et al., 2013; Hegazy et al., 2021; Kappelmayer & Nagy, 2017; Weinstock et al., 2015; Z. Yang et al., 2015), nomeadamente o CD36, CD49f, CD61, CD62p e CD40.

No caso do CD36, a redução da sua expressão foi associada à recuperação mais rápida e à melhoria funcional após lesão da medula espinhal (Myers et al., 2014) e o seu aumento tem sido relacionado com a inflamação e lesões cerebrais (Balkaya et al., 2021; Puchałowicz & Rać, 2020). Na nossa investigação, nos doentes com dor crónica observámos redução da expressão de CD36. No entanto, apesar da redução deste CD estar significativamente associada à existência de dor crónica, foi ainda observado uma redução significativa do número de plaquetas que expressam CD36 nos doentes sob

utilização de opióides. Estes resultados sugerem que a redução do CD36 poderá constituir um biomarcador de resposta aos opióides. No entanto, não foi possível associar a diminuição da expressão desta proteína membranar com a diminuição da presença da dor.

Relativamente ao CD49f, a redução da sua expressão tem sido associada com a diminuição de processos inflamatórios (Yang et al., 2015) e neoplásicos (Ammothumkandy et al., 2016). Na nossa investigação, a redução da expressão de CD49f está significativamente associada à existência de dor crónica e foi ainda observada uma redução significativa da sua expressão na utilização terapêutica de opióides.

Quanto ao CD61, os dados disponíveis são pouco consistentes, visto que tanto o aumento da sua expressão parece estar associada à rápida melhoria da dor em doentes com enfarte agudo do miocárdio (Ribeiro et al., 2022), como a utilização de anticorpos monoclonais anti-CD61 foi associada a melhoria da funcionalidade e dor em doentes pós-enfarte agudo do miocárdio (Carubbi et al., 2019). Na nossa investigação, observámos uma redução do número de plaquetas expressando CD61 e uma redução da expressão desta glicoproteína em doentes com dor crónica, assim como uma redução da sua expressão na presença de opióides, não tendo sido possível associar esta diminuição da expressão com a diminuição da dor.

O aumento da expressão de CD62p já foi associado à atividade de doenças inflamatórias, como a artrite reumatóide (Qu et al., 2019), a doença arterial coronária (Shen et al., 2020) e a fibrilhação auricular (Choudhury et al., 2007). Na nossa investigação, nos doentes com dor crónica observámos aumento da expressão de CD62p , sendo esta diferença mais significativa nos doentes do sexo masculino, onde o aumento da expressão desta proteína membranar foi de cerca de três vezes superior quando comparado com doentes sem dor. Ao contrário das proteínas membranares referidas anteriormente, não detectámos alterações significativas nos níveis de CD62p nos doentes em tratamento analgésico, particularmente nos doentes tratados com opióides. Por outro lado, verificámos um aumento significativo da expressão de CD62p nos doentes com dor nociceptiva, quando comparados com os doentes com dor neuropática e com dor mista, sugerindo a relevância da avaliação da expressão deste CD plaquetar na identificação do tipo de dor, nomeadamente da dor nociceptiva.

Quanto ao CD40, o aumento da expressão deste receptor da família do TNFα tem sido associado a doenças inflamatórias (Antoniades et al., 2009; Karnell et al., 2019; Senhaji et al., 2015) e aterotrombose (Karnell et al., 2019), para além de lesões do sistema nervoso central e dor neuropática (L. Cao et al., 2009; Ots et al., 2022). Na nossa investigação, não encontramos alterações significativas no CD40 quando comparamos doentes com dor e doentes sem dor, embora se tenha observado um aumento significativo da percentagem de plaquetas que expressam CD40 nos doentes com dor moderada a severa. Estes resultados sugerem que o CD40 poderá ter um papel essencialmente na caracterização da intensidade da dor, nomeadamente na deteção de dor moderada a severa. Este resultado pode ser muito relevante e permitir uma terapêutica mais eficaz, nomeadamente em doentes que não expressam a sua dor, como os doentes com demência, nos quais as escalas de avaliação de dor são difíceis de aplicar.

Estando o CD36, o CD49f, o CD61, o CD62p e o CD40 associados à promoção da inflamação e a mecanismos relacionados com a trombose e a apoptose (Barbar et al., 2020; L. Cao et al., 2009; Daviet & McGregor, 1997; Hegazy et al., 2021; Karnell et al., 2019; Ots et al., 2022; Puchałowicz & Rać, 2020; Qu et al., 2019; Senhaji et al., 2015; Z. Yang et al., 2015), os nossos resultados apontam para a multidimensão da dor, e para a multiplicidade de mecanismos que conduzem à sua cronificação. Além disso, apontam para a diversidade de mecanismos associados à nocicepção, para além da variabilidade individual, sendo de destacar a dificuldade de abordagem da influência das patologias dos doentes, particularmente do tipo de demência, na estimulação e/ou inibição da dor.

Assim, relativamente ao estudo laboratorial envolvendo as plaquetas, somos de opinião que o CD36, o CD49f, a percentagem de CD49f, o CD61, a percentagem de CD61 e o CD62p plaquetários são candidatos a biomarcadores de dor, permitindo a atuação célere em cenários em que existe dificuldade no diagnóstico deste sintoma ou doença, sobretudo em doentes com demência ou impossibilitados de comunicar, por outra razão (Ribeiro, et al., 2023).

O CD40 plaquetário poderá vir a ser considerado um biomarcador periférico para a identificação de dor moderada a severa, pelo que o aumento da sua concentração

poderá significar a necessidade de utilização de opióides, segundo a escada analgésica da OMS (Ribeiro, et al., 2023).

Finalmente, o CD62p plaquetário poderá vir a ser considerado um biomarcador periférico de dor nociceptiva, permitindo um ajuste terapêutico relativamente aos adjuvantes utilizados no tratamento farmacológico da dor (Ribeiro et al., 2023).

BIOMARCADORES DA DOR – O PAPEL DOS MONÓCITOS

A mielopoiese, particularmente a proliferação e diferenciação dos monócitos, é estimulada por citocinas pró-inflamatórias, como a IL-3 e IL-6 (Jagannathan-Bogdan & Zon, 2013), o que pode justificar o aumento dos monócitos em muitas condições patológicas que condicionem dor crónica.

Existem três populações diferentes de monócitos, , de acordo com a sua expressão de CD14 e CD16, os monócitos clássicos, os não clássicos e os intermediários, os quais possuem atividades distintas na inflamação (Kapellos et al., 2019; Marimuthu et al., 2018).

Os monócitos têm sido relacionados com várias patologias e a dor de características inflamatórias (Auffray et al., 2009; Taylor et al., 2016), a insuficiência cardíaca (Charach et al., 2019), a dor neuropática (Oggero et al., 2022; Ritz et al., 2011) e a dor crónica inespecífica (Goehler et al., 2014; Taylor et al., 2016). Os monócitos intermediários têm sido associados a doenças inflamatórias (Kapellos et al., 2019; Marimuthu et al., 2018) e os monócitos totais a várias patologias cujo principal sintoma é a dor (Goehler et al., 2014; Ożańska et al., 2020; Taylor et al., 2016).

Foi avaliado o impacto da dor nos valores de monócitos totais, nas populações de subtipos de monócitos, e nas proteínas transmembranares CD11c, CD86, CD163 e CD206, que estão relacionados com várias condições, incluindo a resposta imunitária, a inflamação e a resposta à infeção (Auffray et al., 2009; Espinoza & Emmady, n.d.; Moura, 2011; Santoni et al., 2015).

O CD11c, é uma proteína membranar que tem sido associada a dor inflamatória (Raymondi Silva et al., 2022; Sadhu et al., 2007; Stuhlmüller et al., 2010; Wu et al., 2018) e a dor neuropática (Kohno et al., 2022; Mayrhofer et al., 2021; Tsuda et al., 2023). Segundo os nossos resultados, não foram observadas diferenças significativas na expressão de CD11c na presença ou ausência de dor. No entanto, a percentagem de monócitos expressando CD11c e os níveis desta proteína estão significativamente aumentados na dor mista. Realça-se que nos monócitos não clássicos apenas esta proteína se encontra aumentada na presença de dor mista. Estes dados estão de acordo com a literatura (Mayrhofer et al., 2021; Sadhu et al., 2007; Stuhlmüller et al., 2010; Wu et al., 2018), que relaciona o CD11c com a dor nociceptiva e a dor neuropática, sendo de salientar que apesar dos monócitos não clássicos ainda não apresentarem as suas funções totalmente esclarecidas, estão associados à cronificação dos processos inflamatórios. Portanto, seria expectável que o CD11c estivesse aumentado nos doentes com dor crónica (Mayrhofer et al., 2021; Stuhlmüller et al., 2010). No entanto, há que considerar a multiplicidade de mecanismos que conduzem à cronificação da dor, sem o envolvimento de inflamação crónica, e à diversidade de mecanismos associados à nocicepção, para além da variabilidade individual. Destaca-se ainda a dificuldade de abordagem da influência das patologias dos doentes, particularmente do tipo de demência, na estimulação e na inibição da dor.

Relativamente ao CD86, esta proteína transmembranar tem sido associada à dor neuropática (Kobiela Ketz et al., 2016; Lee et al., 2018; Rutkowski et al., 2004). No nosso estudo, não foram encontradas alterações significativas desta proteína transmembranar relativamente à presença ou ausência de dor, à intensidade da dor ou ao tipo de dor crónica apresentada pelos doentes. Estas diferenças poderão estar relacionadas com o facto de na nossa amostra termos um número reduzido de doentes com dor neuropática.

Quanto ao CD163, esta proteína tem sido associada à resposta imunitária e à atividade de doenças inflamatórias, como a artrite reumatóide, várias infeções e neoplasias malignas (Kowal et al., 2011; Moura, 2011; Pey et al., 2014; Roberts et al., 2004; Tippett et al., 2011), já tendo sido publicados estudos que a associam à dor inflamatória (Kowal et al., 2011; Ohashi et al., 2022) e à dor neuropática (Niehaus et al., 2021). Na nossa

investigação, a expressão de CD163 nos monócitos totais e nos clássicos estava significativamente diminuída em doentes com dor crónica e nos monócitos clássicos a expressão de CD163 estava significativamente reduzida nos doentes com dor nociceptiva, não existindo diferenças nos monócitos intermediários. Estes resultados não estão de acordo com a literatura (Kowal et al., 2011; Ohashi et al., 2022), que mostram um aumento da expressão deste CD na dor crónica, particularmente nos monócitos intermediários. De salientar a diversidade de mecanismos conhecidos e associados à nocicepção, para além da variabilidade individual fisiológica e fisiopatológica, que tem que ser considerada na estimulação e na inibição da dor, assim como na sua cronificação (Raffaeli et al., 2021).

Além disso, nos monócitos de doentes submetidos a tratamento com opióides, observámos uma diminuição da expressão de CD163 , enquanto que nos doentes sob paracetamol se observou aumento da expressão deste marcador, sobretudo em monócitos não clássicos. Estes resultados sugerem a eventual relevância do CD163 na avaliação da resposta à terapêutica da dor, estando a variação dos níveis deste CD dependentes do tipo de fármaco utilizado (opióide vs. paracetamol).

No caso do CD206, esta proteína membranar tem sido associada à resposta imunitária inata e adaptativa (Holder et al., 2014; Tanaka et al., 2021; Tsuchiya et al., 2019) e a dor inflamatória (van der Vlist et al., 2022) e dor neuropática (Chen et al., 2022; Kiguchi et al., 2015; Silva et al., 2022). Na nossa investigação, observámos um aumento dos monócitos que expressam CD206 nos doentes com dor crónica, mas nos monócitos clássicos e nos totais destes doentes, a expressão de CD206 estava significativamente diminuída. Além disso, nos monócitos clássicos, há uma redução significativa do *ratio* CD163/CD206 nos doentes com dor crónica. Nos doentes com dor nociceptiva observámos um aumento significativo na expressão de CD206 nos monócitos intermediários e clássicos e do *ratio* CD163/CD206. Estes resultados estão de acordo com o que foi encontrado por outros investigadores (Kowal et al., 2011; Moura, 2011; Ohashi et al., 2022; Roberts et al., 2004; Silva et al., 2022; Tanaka et al., 2021) e salientam a relevância dos subtipos de monócitos e dos seus níveis de CD206 e da razão CD163/CD206 na caracterização do tipo de dor.

Assim, a concentração relativa de monócitos, particularmente de monócitos clássicos, assim como o aumento de CD206 e a diminuição do *ratio* CD163/CD206 podem vir a ser considerados biomarcadores de dor. A avaliação do CD11c, CD163 e CD206 nos monócitos clássicos, podem vir a ser considerados biomarcadores de dor nociceptiva (Ribeiro, et al., 2023).

A redução da expressão de CD163 em monócitos intermédios em doentes submetidos a terapêutica com opióides corrobora a evidência do poder anti-inflamatório dos opióides através da inativação monocitária (Ribeiro et al., 2023).

APOPTOSE – NOVOS ALVOS TERAPÊUTICOS E POTENCIAIS BIOMARCADORES DE DOR

Um dos contributos importantes desta tese foi também perceber de que forma a apoptose poderá estar implicada na dor, particularmente na dor neuropática (Ribeiro et al., 2022) e identificar potenciais alvos terapêuticos. Foram identificados vários padrões de dor neuropática, que pode ser causada por sensibilização central ou periférica, alterações no *Transient Receptor Potential* (TRP), nos canais de sódio e de cálcio, nas fibras aferentes primárias e distúrbios de sinal excitatório vs. inibitório (Bosma et al., 2018; Finnerup et al., 2021; Jones et al., 2004; Serra et al., 2012; Teixeira-Santos et al., 2020), pelo que o tratamento poderá um dia ser dirigido a estas condições e não estar apenas dependente da caracterização da dor do doente, com uma generalização de fármacos previstos na presença de determinado tipo de dor.

Alguns estudos apontam para que o NF-KB, as citocinas pró-inflamatórias como o TNF-α e o stresse oxidativo tenham um papel importante na dor neuropática periférica, através da modulação da apoptose (Deng et al., 2020; Dostert et al., 2019; Gloire et al., 2006; Sandoval et al., 2018; Teixeira-Santos et al., 2020).

Sendo as mitocôndrias o local de maior produção de espécies reactivas de oxigénio (ROS) e também o alvo da lesão oxidativa que pode conduzir à apoptose, a adopção de medidas para prevenir e proteger a função mitocondrial pode constituir uma estratégia terapêutica promissora para aliviar ou prevenir condições de dor crónica (Muscoli et al., 2013; Schoenberger et al., 2008). Assim, moduladores do TNF-α, inibidores do

proteasoma, inibidores da apoptose (através da modulação do BCL-2 e da caspase-3), e anti-oxidantes, como os flavonóides, entre outros, podem ter potencial para tratar a dor neuropática (Leng et al., 2012; Liao et al., 2022).

Desta forma, somos de opinião que a apoptose e as várias vias de sinalização celular envolvidas neste processo, poderão conduzir a que no futuro próximo sejam encontrados biomarcadores para a identificação e a caracterização da dor, assim como poderão ser produzidos novos fármacos que, inibindo a apoptose, potenciem o tratamento da dor crónica.

LIMITAÇÕES

As principais limitações dos estudos laboratoriais é a amostra reduzida e heterogénea (doentes com diferentes tipos de demência e evoluções temporais da dor), a ausência de igualdade entre idades, sexo e outras características para controlo, não tendo igualmente uma igualdade populacional entre o grupo com dor e com demência face aos controlos (com dor sem demência, sem dor com demência e sem dor e sem demência).

Por outro lado, os estudos laboratoriais não contemplaram pessoas saudáveis, tendo apenas incluído doentes em seguimento por uma equipa de cuidados paliativos domiciliária, ou seja, em situação de grande fragilidade, o que pode contribuir para enviesamentos nos resultados obtidos.

Tratando-se de estudos transversais, não foi possível avaliar se os biomarcadores identificados teriam alterações com novas terapêuticas nem durante o respectivo *follow-up* do tratamento da dor. Também não foi possível avaliar todas as terapêuticas instituídas, que podem eventualmente desenvolver importantes vieses para as conclusões obtidas.

Relativamente às revisões dos opióides e dos AINEs, salienta-se que os trabalhos não se centraram em estudos comparativos entre os vários fármacos disponíveis em Portugal, o que poderia atestar as relações apontadas de maior ou menor segurança em algumas populações especiais. Tal deve-se ao facto de não haver suficientes estudos

comparativos e os que existem apresentarem marcada heterogeneidade metodológica. Outra limitação importante é não termos comparação da eficácia entre os medicamentos opióides, incluindo nos diferentes tipos de dor, sendo que no caso dos AINEs os estudos apontaram para uma equivalência entre os fármacos estudados quanto ao seu efeito.

PONTOS FORTES E PRINCIPAIS CONCLUSÕES

Os trabalhos de revisão, apesar de não se apresentarem como revisões sistemáticas, procuraram atribuir níveis de evidência e forças de recomendação para as principais conclusões obtidas.

As alterações corporais e de funcionalidade orgânica, nomeadamente hepática e renal, possuem uma influência significativa na farmacocinética dos fármacos mais prescritos para o tratamento da dor, como o paracetamol, os AINEs e os opióides.

Os fármacos hidrossolúveis obrigam habitualmente a uma redução da dose, enquanto os fármacos lipossolúveis obrigam a um ajuste da posologia (menos administrações diárias se o doente é obeso ou idoso). Por outro lado, os fármacos com grande eliminação renal obrigam a ajustes posológicos e de dose. Finalmente, os fármacos com metabolização hepática na fase 1 de biotransformação devem ser substituídos sempre que possível por fármacos com metabolização hepática na fase 2 ou ajustadas as posologias para administrações mais espaçadas e com doses menores.

Quanto aos estudos laboratoriais, sendo pioneiros na utilização dos biomarcadores plaquetários e monocitários em doentes com necessidade de equipa especializada em cuidados paliativos, apresenta-se como o maior estudo do género de que temos conhecimento.

Vários potenciais biomarcadores de dor foram identificados no sangue periférico, nomeadamente em plaquetas e monócitos. Salienta-se o aumento da percentagem de plaquetas que expressa CD62p e da percentagem de monócitos, particularmente da subpopulação de monócitos clássicos e da expressão de CD206 monocitária, bem como

a diminuição da percentagem de plaquetas que expressam CD36, CD49f e CD61 e dos níveis de expressão de CD49f e CD61 plaquetários.

Além disso identificámos potenciais biomarcadores periféricos de caracterização da intensidade e tipo de dor, sendo de salientar o CD40 plaquetário na identificação de dor moderada-severa, o CD62 plaquetário e o CD163 e CD206 monocitários na identificação da dor nociceptiva e o CD11c dos monócitos na caracterização da dor mista. Estes resultados poderão contribuir para um melhor ajuste terapêutico dos fármacos utilizados no tratamento farmacológico da dor, contribuindo para um controlo mais rápido e adequado da dor e redução do sofrimento associado ao doente com dor.

A descoberta de novos biomarcadores de dor por métodos não invasivos e independentes da participação do doente, pode contribuir para a identificação de novos alvos terapêuticos e para o desenvolvimento de fármacos mais eficazes e direcionados para o tratamento da dor crónica, com particular relevância nos doentes em cuidados paliativos com demência grave.

O esclarecimento dos mecanismos da aptoptose poderão levar à descoberta de novos biomarcadores de dor e de novas terapêuticas dirigidas. Por outro lado, a detecção/identificação de biomarcadores de dor por métodos não invasivos e independentes da participação do doente, pode contribuir para a identificação de novos alvos terapêuticos e para o desenvolvimento de fármacos mais eficazes e direcionados para o tratamento da dor crónica, com particular relevância nos doentes em cuidados paliativos com demência grave.

PERSPETIVAS FUTURAS

São, no futuro próximo, necessários estudos confirmativos, multicêntricos, com amostras maiores, que comparem populações diferentes, com diferentes causas de dor, diferentes mecanismos para a demência, diferentes respostas aos tratamentos instituídos, diferentes evoluções temporais da dor. Outras características, como a localização, fatores predisponentes e de melhoria, sintomas associados e outros factores também deverão ser estudados e esclarecidos.

Com uma coorte de doentes como o que foi enunciado e numa perspectiva prospectiva, poderemos confirmar as hipóteses que avançámos com os estudos laboratoriais.

Quanto aos perfis de farmacocinética e farmacodinâmica, são necessários mais estudos, nomeadamente ensaios clínicos aleatorizados, duplamente cegos, centrados em características especiais dos doentes (função renal, função hepática, polimedicação, biótipo, massa magra e massa gorda, água corporal), comparando os diferentes AINEs e os diferentes opióides em que se centraram estes trabalhos, a fim de serem melhorados os níveis de evidência e as forças de recomendação apresentadas nestes trabalhos. Para além disso, são necessários estudos que adicionem a farmacogenómica às variáveis em estudo, pois a sua influência será determinante para a farmacocinética e a farmacodinâmica destes medicamentos, condicionando a abordagem farmacológica individualizada que se pretende para o futuro.

Relativamente à apoptose, já estão em curso vários ensaios clínicos que poderão confirmar muitos dos potenciais biomarcadores e validar novas armas terapêuticas, particularmente para a dor neuropática.

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Anexo 1 – Variáveis

- Data de nascimento

- Data de morte

- Sexo (masculino ou feminino)

- Dor (utilizando a Escala de Avaliação da Dor na Demência Avançada)

- Estado funcional (utilizando o Índice de Karnovsky)

- Estado cognitivo (utilizando a Escala de Deterioração Global)

- Estado físico e nutricional (utilizando a escala *Mini Nutritional Assessment*)

- Função renal (através da Taxa de Filtração Glomerular, medida de acordo com a equação de Cockcroft-Gault)

- Função hepática (através da classificação de Child-Pugh)

- Tipo de demência (Doença de Alzheimer, Demência Vascular, Doença de Parkinson, Demência de Corpos de Lewy, Demência Frontotemporal, Doença de Huntington, Demência provocada pelo álcool e Doença de Creutzfeldt-Jacob)

- Biomarcadores periféricos de dor:

Dados do hemograma: populações leucocitárias, indíces plaquetários (PDW)

Caracterização imunofenotípica/activação das plaquetas e monócitos/macrófagos por citometria de fluxo - expressão de CD40, CD41a, CD42a, CD61, CD62p, CD36, CD49f, e CD59 (plaquetas); CD14, CD16 (monócitos), CD86/CD11c (M1) and CD163/CD206 (M2)

Anexo 2 – Escalas

1- ESCALA DE AVALIAÇÃO DA DOR NA DEMÊNCIA AVANÇADA (PAINAD) (adaptado de Batalha et al., 2012)

Categoria	Item	Pontuação
Respiração independente da vocalização	Normal.	0
	Respiração ocasionalmente difícil. Curto período de hiperventilação.	1
	Respiração difícil e ruidosa. Período longo de hiperventilação. Respiração *Cheyne-Stok*.	2
Vocalização negativa	Nenhuma.	0
	Queixume ou gemido ocasional. Tom de voz baixo com discurso negativo ou de desaprovação.	1
	Chamamento perturbado repetitivo. Queixume ou gemido alto. Choro.	2
Expressão facial	Sorridente ou inexpressiva.	0
	Triste. Amedrontada. Sobrancelhas franzidas.	1
	Esgar facial.	2
Linguagem corporal	Relaxada.	0
	Tensa. Andar para cá e para lá de forma angustiada. Irrequieta.	1
	Rígida. Punhos cerrados. Joelhos flectidos. Resistência à aproximação ou ao cuidado. Agressiva.	2
Consolabilidade	Sem necessidade de consolo.	0
	Distraído ou tranquilizado pela voz ou toque.	1
	Impossível de consolar, distrair ou tranquilizar.	2

2- ÍNDICE DE KARNOVSKY (adaptado de Schag et al., 1984)

Capaz de exercer uma atividade normal e ao trabalho; nenhum cuidado especial é necessário.	100	Normal sem queixas, sem evidência de doença.
	90	Capaz de exercer uma atividade normal, com pequenos sinais ou sintomas da doença.
	80	Atividade normal com esforço, alguns sinais ou sintomas da doença.
Impossibilitado de trabalhar, capaz de viver em casa e cuidar de necessidades mais pessoais; quantidade variável de assistência necessária.	70	Cuidados para si, incapaz de exercer uma atividade normal ou para fazer um trabalho ativo.
	60	Requer assistência ocasional, mas é capaz de cuidar mais de suas necessidades pessoais.
	50	Requer considerável assistência e cuidados médicos frequentes.
Incapaz de cuidar de si mesmo, requer equivalente de cuidados institucionais ou hospitalares; a doença pode estar progredindo rapidamente.	40	Com deficiência; requer cuidados e assistência especiais.
	30	Com deficiência grave, a hospitalização é indicada, embora a morte não era iminente.
	20	Muito doente, a hospitalização necessárias; tratamento de suporte ativo necessário.
	10	Moribundos; processos fatal progredindo rapidamente.
	0	

3- ESCALA DE DETERIORAÇÃO GLOBAL (EDG) (adaptado de Reisberg et al., 1982)

Estadios Cognitivos	Comportamentos
Estadio 1: Nível cognitivo normal	A pessoa não apresenta défice de memória, nem problemas ao realizar tarefas diárias.
Estadio 2: Declínio cognitivo Subjetivo	Demonstra sinais de um declínio cognitivo leve, como falhas de memória.
Estadio 3: Declínio cognitivo ligeiro	Dificuldades no trabalho, pode perder-se, não sabe onde colocou um objeto de valor.
Estadio 4: Demência ligeira	Começa a perder conhecimento sobre si mesmo e eventos a decorrer, tem dificuldades com as finanças ou para preparar uma refeição.
Estadio 5: Demência moderada	Não consegue sobreviver sozinho, dificuldades em relatar detalhes pessoais importantes como a morada.
Estadio 6: Demência moderada/severa	Não consegue relatar acontecimentos que aconteceram há pouco tempo, não sabe o nome das pessoas próximas, incontinência, precisa de ajuda para se vestir. Agitação e problemas de personalidade.
Estadio 7: Demência severa	As palavras percetíveis são escassas, perde a capacidade verbal, vai perdendo a capacidade de andar, de sentar sem ajuda, de sorrir ou de mexer a cabeça.

1- Ausência de queixas subjectivas de défice de memória

Sem défice de memória evidente durante a entrevista

2- Queixas subjectivas de défice de memória, mais frequentemente nas seguintes áreas:

a. Esquecimento do local onde colocou objectos familiares
b. Esquecimento de nomes antes bem conhecidos

Sem défice de memória objectivo durante a entrevista.

Sem défice objectivo no emprego ou ocasiões sociais.

Preocupação legítima em relação aos sintomas.

3- **Ocorrências precoces de défice claro.** Manifestações em mais do que uma das seguintes áreas:
    a. O paciente pode ter-se perdido quando viajava para um local desconhecido
    b. Os colegas dão-se conta do desempenho relativamente pobre do paciente
    c. As dificuldades em encontrar palavras e/ou nomes tornam-se evidentes para as pessoas mais próximas
    d. O paciente pode ler um texto, ou um livro, e reter relativamente pouco
    e. O paciente pode apresentar facilidade diminuída em recordar os nomes de pessoas a quem é apresentado
    f. O paciente pode ter perdido, ou colocado em local errado, um objecto de valor
    g. Os testes clínicos podem evidenciar défice de concentração

    Défice de memória objectiva, evidente **só no decurso duma entrevista intensiva**.

    Diminuição do desempenho em ocupações laborais de maior exigência, e em situações sociais.

    O paciente começa a manifestar negação.

    Ansiedade ligeira a moderada que acompanha frequentemente os sintomas.

4- **Défices claramente evidentes em entrevista clínica cuidadosa.** Défice manifesto nas seguintes áreas:
    a. Decréscimo no conhecimento de acontecimentos correntes e recentes
    b. Pode aresentar algum défice de memória na sua própria história pessoal
    c. Défice de concentração evidenciado nas subtracções seriadas
    d. Diminuição da capacidade para viajar, gerir as finanças, etc

5- **O paciente não consegue sobreviver sem alguma assistência.** Durante a entrevista o paciente é incapaz de se recordar de aspectos relevantes da sua vida actual, por exemplo:
    a. Do seu endereço ou do seu número de telefone, que tem desde há muitos anos
    b. Dos nomes dos seus familiares mais próximos (como os seus netos)
    c. Do nome do liceu (escola secundária) ou da faculdade onde se formou

    Apresenta frequentemente alguma desorientação relativamente ao tempo (data, dia da semana, estação do ano, etc) ou ao local

    Uma pessoa escolarizada pode ter alguma dificuldade na subtracção em séries de 4 em 40 ou de 2 em 20

    As pessoas neste nível retêm a lembrança dos factos mais importantes relativamente a si próprios, e a outros

    Sabem invariavelmente os seus próprios nomes, e conhecem, geralmente, os nomes do cônjuge e dos filhos

    Não precisam de auxílio para os cuidados de higiene, nem para as refeições, mas podem ter dificuldade na escolha da roupa adequada para vestir.

    É frequente continuarem a ser capazes de distinguir, no meio em que vivem, as pessoas conhecidas das não conhecidas.

    Ocorrem alterações emocionais e da personalidade. São muito variáveis e incluem:

        i. Comportamento delirante, isto é, o paciente pode acusar o seu cônjuge de ser uma pessoa impostora, pode falar para figuras imaginárias à sua volta, ou para a sua própria imagem reflectida no espelho
        ii. Sintomas obsessivos, isto é, a pessoa pode executar, repetida e sistematicamente, actividades simples de limpeza
        iii. Podem ocorrer sintomas de ansiedade, agitação, e mesmo um comportamento violento antes inexistente
        iv. Abolia cognitiva, isto é, perda de força de vontade, por o indivíduo não conseguir manter um pensamento até completar uma acção

6- Podem ocasionalmente esquecer-se do nome do conjuge de quem dependem inteiramente para sobreviver.

Podem de todo **não se aperceber de todos os acontecimentos e experiências recentes das suas vidas.**

Retêm alguma noção do que os rodeia, do ano, da estação do ano, etc.

Podem ter dificuldade em contar de 1 até 10, em ordem decrescente e, por vezes, em ordem crescente

Necessitarão de alguma assistência nas actividades de vida diária:

a. Podem ficar incontinentes
b. Necessitarão de assistência para viajar, mas poderão, ocasionalmente, ser capazes de viajar para locais conhecidos

O ritmo diurno encontra-se frequentemente alterado.

Lembram-se quase sempre do seu próprio nome.

7- Ao longo deste nível vão sendo perdidas todas as faculdades verbais.

Na fase inicial deste nível as palavras e as frases são faladas, mas o discurso é muito limitado. Mais tarde deixa de haver discurso – apenas sons grunhidos.

Incontinente; requer assistência na higiene e na alimentação.

As competências psicomotoras básicas (como por exemplo a capacidade de andar) vão sendo perdidas com a progressão deste nível.

O cérebro parece não ser mais capaz de comandar o corpo.

Presença frequente de sinais e sintomas neurológicos difusos e corticais.

4- MININUTRITIONAL ASSESSMENT (MNA) (adaprado de Duque et al., 2000)

Sobrenome: Nome: Sexo: Data:

Idade: Peso (kg): Altura (cm): Leito:

Preencher a primeira parte deste questionário, indicando a resposta. Somar os pontos da Triagem. Caso o escore seja igual ou inferior a 11, concluir o questionário para obter a avaliação do estado nutricional.

Triagem

A Nos últimos três meses houve diminuição da ingesta alimentar devido a perda de apetite, problemas digestivos ou dificuldade para mastigar ou deglutir?
0 = diminuição severa da ingesta
1 = diminuição moderada da ingesta
2 = sem diminuição da ingesta ☐

B Perda de peso nos últimos meses
0 = superior a três quilos
1 = não sabe informar
2 = entre um e três quilos
3 = sem perda de peso ☐

C Mobilidade
0 = restrito ao leito ou à cadeira de rodas
1 = deambula mas não é capaz de sair de casa
2 = normal ☐

D Passou por algum estresse psicológico ou doença aguda nos últimos três meses?
0 = sim 2 = não ☐

E Problemas neuropsicológicos
0 = demência ou depressão graves
1 = demência leve
2 = sem problemas psicológicos ☐

F Índice de massa corpórea (IMC = peso [kg] / estatura [m]^2)
0 = IMC < 19
1 = 19 ≤ IMC < 21
2 = 21 ≤ IMC < 23
3 = IMC ≥ 23 ☐

Escore de triagem (subtotal, máximo de 14 pontos) ☐☐

12 pontos ou mais — normal; desnecessário continuar a avaliação

11 pontos ou menos — possibilidade de desnutrição; continuar a avaliação

Avaliação global

G O paciente vive em sua própria casa (não em casa geriátrica ou hospital)
0 = não 1 = sim ☐

H Utiliza mais de três medicamentos diferentes por dia?
0 = sim 1 = não ☐

I Lesões de pele ou escaras?
0 = sim 1 = não ☐

J Quantas refeições faz por dia?
0 = uma refeição
1 = duas refeições
2 = três refeições ☐

K O paciente consome:
- pelo menos uma porção diária de leite ou derivados (queijo, iogurte)? sim ☐ não ☐
- duas ou mais porções semanais de legumes ou ovos? sim ☐ não ☐
- carne, peixe ou aves todos os dias? sim ☐ não ☐

0,0 = nenhuma ou uma resposta «sim»
0,5 = duas respostas «sim»
1,0 = três respostas «sim» ☐,☐

L O paciente consome duas ou mais porções diárias de frutas ou vegetais?
0 = não 1 = sim ☐

M Quantos copos de líquidos (água, suco, café, chá, leite) o paciente consome por dia?
0,0 = menos de três copos
0,5 = três a cinco copos
1,0 = mais de cinco copos ☐,☐

N Modo de se alimentar
0 = não é capaz de se alimentar sozinho
1 = alimenta-se sozinho, porém com dificuldade
2 = alimenta-se sozinho sem dificuldade ☐

O O paciente acredita ter algum problema nutricional?
0 = acredita estar desnutrido
1 = não sabe dizer
2 = acredita não ter problema nutricional ☐

P Em comparação a outras pessoas da mesma idade, como o paciente considera a sua própria saúde?
0,0 = não muito boa
0,5 = não sabe informar
1,0 = boa
2,0 = melhor ☐,☐

Q Circunferência do braço (CB) em cm
0,0 = CB < 21
0,5 = 21 ≤ CB ≤ 22
1,0 = CB > 22 ☐,☐

R Circunferência da panturrilha (CP) em cm
0 = CP < 31 1 = CP ≥ 31 ☐

Avaliação global (máximo 16 pontos) ☐☐,☐
Escore da triagem ☐☐
Escore total (máximo 30 pontos) ☐☐,☐

Avaliação do Estado Nutricional

de 17 a 23,5 pontos — risco de desnutrição ☐
menos de 17 pontos — desnutrido ☐

Ref.: Guigoz Y. Vellas B and Garry PJ. 1994. Mini Nutritional Assessment: A practical assessment tool for grading the nutritional state of elderly patients. *Facts and Research in Gerontology*. Supplement #2:15-59.
Rubenstein LZ, Harker J, Guigoz Y and Vellas B. Comprehensive Geriatric Assessment (CGA) and the MNA: An Overview of CGA, Nutritional Assessment, and Development of a Shortened Version of the MNA. In: "Mini Nutritional Assessment (MNA): Research and Practice in the Elderly". Vellas B, Garry PJ and Guigoz Y, editors. Nestlé Nutrition Workshop Series. Clinical & Performance Programme, vol. 1. Karger, Bâle, in press.

Anexo 3 – Consentimento informado aos doentes

A partir da página seguinte.

ANEXO 3 - FORMULÁRIO DE INFORMAÇÃO E CONSENTIMENTO INFORMADO

TÍTULO DO PROJECTO DE INVESTIGAÇÃO:

Controlar a Dor em doentes com demência avançada: o papel dos biomarcadores na caracterização da dor e a importância da farmacocinética no apoio à decisão terapêutica

PROTOCOLO Nº:	Não aplicável
PROMOTOR (Entidade ou pessoa(s) que propõe(m) o estudo):	- Hugo Alexandre da Cruz Ribeiro - José Paulo Andrade - Marília de Assunção Rodrigues Ferreira Dourado - Manuel Teixeira Marques Veríssimo
INVESTIGADOR COORDENADOR	Hugo Alexandre da Cruz Ribeiro
CENTRO DE ESTUDO	

INVESTIGADOR PRINCIPAL Hugo Alexandre da Cruz Ribeiro

MORADA Rua Barão do Corvo, 676, 4400-037 Vila Nova de Gaia

CONTACTO TELEFÓNICO 223747010

NOME DO DOENTE

(LETRA DE IMPRENSA) _____

NOME DO CUIDADOR

(LETRA DE IMPRENSA) _____

É convidado(a) a participar neste estudo porque é um doente em tratamento na Consulta da Equipa Comunitária de Suporte em Cuidados Paliativos de Gaia.

Este procedimento é chamado consentimento informado e descreve a finalidade do estudo, os procedimentos, os possíveis benefícios e riscos. A sua participação poderá contribuir para melhorar o conhecimento sobre a dor, melhorando a abordagem médica no que diz respeito à sua avaliação e tratamento.

Receberá uma cópia deste Consentimento Informado para rever e solicitar aconselhamento de familiares e amigos. O Investigador ou outro membro da sua equipa irá esclarecer qualquer dúvida que tenha sobre o termo de consentimento e também alguma palavra ou informação que possa não entender.

Depois de compreender o estudo e de não ter qualquer dúvida acerca do mesmo, deverá tomar a decisão de participar ou não. Caso queira participar, ser-lhe-á solicitado que assine e date este formulário. Após a sua assinatura e a do Investigador, ser-lhe-á entregue uma cópia. Caso não queira participar, não haverá qualquer penalização nos cuidados que irá receber.

1. INFORMAÇÃO GERAL E OBJECTIVOS DO ESTUDO

Este estudo irá decorrer na Equipa Comunitária de Suporte em Cuidados Paliativos de Gaia, em colaboração com a Faculdade de Medicina da Universidade do Porto, com o objectivo de avaliar a presença de biomarcadores de dor que possibilitem a sua caracterização e consequente abordagem terapêutica mais dirigida, assim como estudo de farmacogenética, possibilitando a antevisão de efeitos dos medicamentos em cada doente, aumentando a eficácia e a segurança dos tratamentos. O estudo permitirá ainda avaliar a melhoria do estado geral dos utentes com o controlo da dor.

Trata-se de um estudo observacional, pelo que não será feita nenhuma alteração na sua medicação ou tratamentos habituais devido ao mesmo.

Este estudo foi aprovado pela Comissão de Ética da Faculdade Medicina da Universidade do Porto (FMUP) e pela Comissão de Ética da Administração Regional de Saúde do Norte de modo a garantir a protecção dos direitos, segurança e bem-estar de todos os doentes ou outros participantes incluídos e garantir prova pública dessa protecção.

2. PROCEDIMENTOS E CONDUÇÃO DO ESTUDO

2.1. Procedimentos

A avaliação do estado cognitivo, mental, nutricional e físico, funcional dos doentes é realizada através dos registos das consultas que o doente tem no âmbito do seu seguimento na Consulta da Equipa Comunitária de Suporte em Cuidados Paliativos (ECSCP) de Gaia.

Outras avaliações, nomeadamente a função renal, cardíaca e hepática são avaliadas através de análises sanguíneas que os doentes já fariam com a regularidade adequada a cada situação.

No âmbito deste estudo, pretende efectuar-se uma única colheita de sangue, que será enviada para o Laboratório de Oncobiologia e Hematologia da Faculdade de Medicina da Universidade de Coimbra, onde serão avaliados biomarcadores nociceptivos e polimorfismos genéticos relacionados com a farmacocinética dos fármacos utilizados no tratamento da dor. A amostra ficará guardada durante 5 anos, após os quais será destruída, de acordo com o protocolo de atuação do Laboratório.

2.2. Calendário das visitas/ Duração (exemplo)

Este estudo tem a duração de 3 anos, sendo que serão aproveitados todos os registos das visitas da ECSCP de Gaia.

3. RISCOS E POTENCIAIS INCONVENIENTES PARA O DOENTE

A inclusão dos doentes no estudo não acarreta nenhum risco nem inconveniente.

4. POTENCIAIS BENEFÍCIOS

Este estudo tem a vantagem de estudar a dor de forma individualizada e permitir um melhor conhecimento da dor nos doentes com demência, de modo a melhorar o seu tratamento e evitar efeitos adversos. Além disso, a informação que será recolhida irá contribuir para uma melhor informação dos médicos de forma a melhorar os cuidados clínicos a prestar aos doentes com situações de dor idênticas à sua ou à da pessoa pela qual é responsável pelas decisões relativamente a cuidados de saúde.

7. SEGURANÇA

A participação dos doentes neste estudo não acarreta nenhum risco.

8. PARTICIPAÇÃO/ ABANDONO VOLUNTÁRIO

É inteiramente livre de aceitar ou recusar participar neste estudo. Pode retirar o seu consentimento em qualquer altura sem qualquer consequência para si, sem precisar de explicar as razões, sem qualquer penalidade ou perda de benefícios e sem comprometer a sua relação com o Investigador que lhe propõe a participação neste estudo. Ser-lhe-á pedido para informar o Investigador se decidir retirar o seu consentimento.

9. CONFIDENCIALIDADE

Os seus registos manter-se-ão confidenciais e anonimizados de acordo com os regulamentos e leis aplicáveis. Se os resultados deste estudo forem publicados a sua identidade manter-se-á confidencial.

Ao assinar este Consentimento Informado autoriza este acesso condicionado e restrito.

Ao assinar este termo de consentimento informado, permite que as suas informações médicas neste estudo sejam verificadas, processadas e relatadas conforme for necessário para finalidades científicas legítimas.

11. CONTACTOS

Se tiver perguntas relativas aos seus direitos como participante deste estudo, deve contactar:

Presidente da Comissão de Ética da FMUP,

Alameda Prof. Hernâni Monteiro, 4200-319 Porto

Endereço electrónico institucional (comissao.etica@chsj.min-saude.pt);

Telefone directo (225512126)

Se tiver questões sobre este estudo deve contactar:

Hugo Alexandre da Cruz Ribeiro

hribeiroff@gmail.com

ECSCP Gaia / USF Barão do Corvo

Rua Barão do Corvo, nº 676, 4400-037 Vila Nova de Gaia

TELEFONE: 223747010

CONSENTIMENTO INFORMADO

De acordo com a Declaração de Helsínquia da Associação Médica Mundial e suas actualizações:

1. Declaro ter lido este formulário e aceito de forma voluntária participar neste estudo.
2. Fui devidamente informado(a) da natureza, objectivos, riscos, duração provável do estudo, bem como do que é esperado da minha parte.
3. Tive a oportunidade de fazer perguntas sobre o estudo e percebi as respostas e as informações que me foram dadas.

   A qualquer momento posso fazer mais perguntas ao médico responsável do estudo. Durante o estudo e sempre que quiser, posso receber informação sobre o seu desenvolvimento. O médico responsável dará toda a informação importante que surja durante o estudo que possa alterar a minha vontade de continuar a participar.
4. Aceito que utilizem a informação relativa à minha história clínica e os meus tratamentos no estrito respeito do segredo médico e anonimato. Os meus dados serão mantidos estritamente confidenciais. Autorizo a consulta dos meus dados apenas por pessoas designadas pelo promotor e por representantes das autoridades reguladoras.
5. Aceito seguir todas as instruções que me forem dadas durante o estudo. Aceito em colaborar com o médico e informá-lo(a) imediatamente das alterações do meu estado de saúde e bem-estar e de todos os sintomas inesperados e não usuais que ocorram.
6. Autorizo o uso dos resultados do estudo para fins exclusivamente científicos e, em particular, aceito que esses resultados sejam divulgados às autoridades sanitárias competentes.
7. Aceito que os dados gerados durante o estudo sejam informatizados pelo promotor ou outrem por si designado.

Eu posso exercer o meu direito de rectificação e/ ou oposição.

8. Tenho conhecimento que sou livre de desistir do estudo a qualquer momento, sem ter de justificar a minha decisão e sem comprometer a qualidade dos meus cuidados médicos. Eu tenho conhecimento que o médico tem o direito de decidir sobre a minha saída prematura do estudo e que me informará da causa da mesma.
9. Fui informado que o estudo pode ser interrompido por decisão do investigador, do promotor ou das autoridades reguladoras.

Nome do Participante/Cuidador

_____

Assinatura : _____

Data:_____/_____/____

Confirmo que expliquei ao participante acima mencionado a natureza, os objectivos e os potenciais riscos do Estudo acima mencionado.

Nome do

Investigador:_____

Assinatura: _____

Data:_____/_____/____

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