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The Challenges of Setting Up a Clinical Study with the New European Union Medical Device Regulation

Os Desafios da Implementação de um Estudo Clínico com o Novo Regulamento de Dispositivos Médicos da União Europeia

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Palavras-chave: Investigação Clínica; Legislação de Dispositivos Médicos; União Europeia

INTRODUCTION

The new European Union Medical Device Regulation (MDR) – Regulation EU 2017/745, which came into effect on May 26, 2021, expands the scope of medical devices to "any instrument, apparatus, appliance, software, implant, reagent, material or other article intended by the manufacturer to be used, alone or in combination, for human beings for one or more of the following specific medical purposes: diagnosis, prevention, monitoring, prediction, prognosis, treatment or alleviation of disease; diagnosis, monitoring, treatment, alleviation of, or compensation for, an injury or disability; (…)" (MDR – Article 2),[1] and therefore has had a significant impact in the medical devices industry.

The Apple watch, which analyses heart rate and may potentially detect arrhythmias, was classified as a medical device.[2] Glooko and myDario,[2] which collect and analyze data on a person's diet for the management of diabetes, also received the same classification. Smartphone apps that acquire medical images to enable doctors to diagnose diseases (e.g., pictures of the skin to assess for malignant or benign skin lesions), software that predicts the risk of developing stroke or heart disease, and clinical decision support systems, all became software devices.[2]

According to the new EU MDR, digital health technologies, including apps, stand-alone software, or wearable health devices that claim a medical purpose, qualify as medical devices. Manufacturers now need to provide a clinical evaluation that demonstrates the safety, effectiveness, and benefit of the device (MDR – Article 61),[1] and to go through *conformité européenne* (CE) marking clearance (MDR – Article 10).[1] Moreover, software intended to provide information that is used to make medical decisions qualifies at least as class IIa (MDR – Rule 11 of Annex VIII),[1] which means that it must be assessed by a notified body – an organisation designated by an EU country to assess the conformity of certain medical devices with EU legislation before being placed on the market [MDR – (60)].[1]

Among the changes introduced by the MDR, some aspects pose new challenges in setting up a clinical study with medical software and will be described in the next sections.

CHALLENGES

Understanding the new regulatory requirements

There is currently a lack of clarity regarding how to comply with the multiple new requirements of the MDR.[3] The regulation itself can be difficult to interpret, and therefore templates and guidelines are being developed to support investigators in their submissions to ethics committees and regulators. Documents such as a template for an application to a Medical Research Ethics Committee (MREC) in the Netherlands for non-CE-marked medical devices[4] and the MDCG 2021-6: Regulation (EU) 2017/745 – Questions and Answers regarding clinical investigation by the Medical Devices Coordination Group Document are available.[5] The information is scattered, and each country is producing guidance for their researchers and companies to implement MDR in their setting. Even the authorities are receiving guidance documents, such as the guide for MREC regarding review of clinical research with medical devices.[6] But a concise guide to conduct a clinical evaluation to fulfil a certification procedure is unavailable. Meanwhile, clinical researchers face challenges in complying with the MDR framework and the new rules for the submission of studies, which may complicate and delay the process of conducting a clinical study.

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More documentation and resources to approve clinical studies

The MDR introduces additional requirements regarding the information to be provided to the medical device ethics committee and the regulator for the purpose of a clinical study approval. In addition to a Clinical Evaluation Plan in conformity with the MDR (Annexes IX, XIV and XV),[1] study sponsors need to present extensive documentation of the device, a commercialization label, a complete risk-benefit assessment matrix, and proof of quality procedures through a quality management system (MDR – Chapter II, and Annexes I and II).[1] The device also needs to be registered in the database of EUDAMED (MDR – Chapter III).[1]

Overall, these new requirements make it harder for researchers to navigate the approval process of a clinical study. If any information is missing, additional time is required to obtain it, thus delaying the start of the study. In Europe, it takes a maximum of 60 days for the approval of new studies and 35 days for each amendment (2001/20/EC directive).[7]

This is a particularly challenging period, as standardised protocols are not yet available to everyone. Moreover, compliance of clinical studies with the MDR begins in the early development phases of medical devices. Therefore, the occurrence of a failure to comply with the MDR in the design and development phase, including benchmark testing, could jeopardize the approval for clinical studies with patients and the projects' timeline. The MDR also expects the compliance with the international standard ISO 14155:2020 Clinical investigation of medical devices for human subjects – Good clinical practice[8] (MDR – Annex XV)[1], as well as independent monitoring and auditing for the clinical study (MDR – Annex XV),[1] which entails additional training and human resources. Furthermore, the Clinical Evaluation Report should include both favourable and unfavourable results of the clinical evaluation, as well as reporting of adverse events (MDR – Annex XIV).[1]

More difficulties in finding clinical centers

The EU MDR categorizes medical devices in one of three categories – class I, II (a or b) or III – based on their risk and intended purpose (MDR – Article 51).[1] Clinical evaluation is mandatory, regardless of the risk classification (MDR – Articles 61 and 62).[1] A significant increase in the number of studies with medical devices is expected because under the previous Medical Devices Directive,[9] non-CE-marked technologies in the market were reclassified by the new MDR to a higher risk category that needs reapproval. This means that manufacturers will not be able to produce those medical devices as of May 2024 unless they already generated clinical evidence to fulfil the MDR requirements. Moreover, researchers and companies will have to deal with competition for the same clinical study sites, with inevitable delays. Recruitment time for clinical study volunteers will increase as well, particularly because the number of researchers conducting studies at clinical sites is typically small, and there may not be enough researchers available to conduct the studies at clinical sites. The number of potential participants is also limited, as recruitment usually relies on the researcher's own list of patients. Although these obstacles are not new,[10] the situation may be unprecedentedly critical given the increase in the number of clinical studies.

Higher costs

The new MDR also mandates the subscription of an insurance policy for clinical studies with medical devices (article 69),[1] similar to those of new drugs. Insurance companies assess the clinical study risk mainly based on three factors: the characteristics of the medical device (risk class, CE-mark or not), the stage of the medical device clinical investigation, and the number of patients.[11] Considering the new classification rules for medical devices introduced by the MDR, study sponsors and researchers may face the challenge of a significant increase in study insurance costs. We should stress that the market for insurance companies in Europe is small and not very competitive, which works against researchers and companies. We estimate that the average cost of a study of a class IIa medical device (i.e., most software for clinical decision support systems) should amount to €15 600. If we take into consideration that research on digital and mobile medical devices is mostly undertaken by research units, academia, and small and medium enterprises, we anticipate that the conduct of studies by this sector could be seriously compromised.

Addressing the challenges

Team-based development of medical devices, bringing together engineers, designers, clinical researchers, and regulatory issues specialists seems to be the best approach to meet the demands of a complex regulatory landscape. Early communication with regulatory authorities facilitates compliance with the EU MDR as well. To advance medical devices towards clinical testing, strategic partnerships with clinical sites will play a critical role. On the academic and industry sides, this is the opportunity to ensure the conduct of clinical studies and demonstrate the clinical effectiveness and relevance of their technologies. On the clinical site front, partnerships can bring innovative, breakthrough medical devices to address unmet needs. Implementing decentralized clinical studies by performing remote data acquisition could decrease the burden on staff at clinical sites – one of the main barriers to entering clinical studies – and reduce costs associated with patient visits to the clinic. Finally, research centers would greatly benefit from insurance

companies creating plans for machine learning and mobile device-based studies.

CONCLUSION

Clinical evaluation of medical devices has become a more challenging field, mainly because of the difficulty in complying with the more stringent requirements set out by the new MDR. It is, however, an essential part of innovation and an assurance of safety and clinical benefit for patients. Technology teams must adopt interdisciplinary processes where researchers engage with regulatory authorities during the design and development phases of medical devices. Decentralized clinical studies combined with alliances with clinical sites could potentially secure clinical testing and further regulatory and market approval of medical devices.

AUTHOR CONTRIBUTIONS

SR: Conceptualization, literature search, draft writing and preparation, approval of the final version.

MDM, FN: Review and editing, approval of the final version.

COMPETING INTERESTS

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MDM declared that no competing interests exist.

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Anticoagulação Oral e Incidência de Acidente Vascular Cerebral Associado a Fibrilhação Auricular em Portugal Continental: Um Estudo de Modelação

Oral Anticoagulation and the Incidence of Stroke Associated with Atrial Fibrillation in Mainland Portugal: A Modelling Study

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RESUMO

Introdução: A fibrilhação auricular é a disritmia persistente mais prevalente, tendo um importante impacto social e económico. O objetivo principal deste estudo foi avaliar a associação entre a utilização de anticoagulantes orais e a incidência de acidente vascular cerebral associado a fibrilhação auricular, em Portugal continental.

Métodos: A base de dados de morbilidade hospitalar foi utilizada para a contabilização dos episódios de internamento com um diagnóstico principal de acidente vascular cerebral e um diagnóstico adicional de fibrilhação auricular, ocorridos durante cada mês do período em análise (janeiro de 2012 a dezembro de 2018), em indivíduos com idade igual ou superior a 18 anos. O número de doentes com registo de fibrilhação auricular presentes nesta base de dados foi utilizado como um *proxy* da prevalência de fibrilhação auricular conhecida. O número de doentes anticoagulados foi estimado a partir das estatísticas das vendas de antagonistas da vitamina K e novos anticoagulantes orais (apixabano, dabigatrano, edoxabano e rivaroxabano) em Portugal continental. Foi realizada uma análise descritiva das variáveis, construindo-se depois modelos auto-regressivos integrados de médias móveis sazonais (*seasonal autoregressive integrated moving average*, SARIMA), com recurso ao *software R*.

Resultados: Ocorreram, em média, 522 (± 57) episódios de acidente vascular cerebral por mês. Verificou-se um aumento gradual do número de doentes anticoagulados, passando de 68 943 para 180 389, por mês. A tendência decrescente no número de episódios verificou-se a partir de 2016, a par da maior utilização dos novos anticoagulantes orais, comparativamente aos antagonistas da vitamina K. O modelo final estimado indicou que o aumento do consumo de anticoagulação oral entre 2012 e 2018 em Portugal continental foi associado a um decréscimo do número de acidentes vasculares cerebrais associados a fibrilhação auricular. Estimou-se que, entre 2016 e 2018, a mudança no tipo de anticoagulação se associou a uma redução de 833 episódios de acidentes vascular cerebrais em doentes com fibrilhação auricular (4,2%).

Conclusão: A anticoagulação oral associou-se à redução da incidência de acidente vascular cerebral em doentes com fibrilhação auricular, em Portugal continental. Esta redução foi mais relevante no período 2016 a 2018, em provável relação com a introdução dos novos anticoagulantes orais.

Palavras-chave: Acidente Vascular Cerebral/prevenção e controlo; Anticoagulantes/uso terapêutico; Fibrilhação Auricular/complicações; Portugal

ABSTRACT

Introduction: Atrial fibrillation is the most prevalent persistent dysrhythmia, contributing to a significant social and economic burden. The main objective of this study was to evaluate the association between oral anticoagulant use and the incidence of stroke associated with atrial fibrillation, in mainland Portugal.

Methods: The number of episodes of inpatient care with a main diagnosis of stroke and an additional diagnosis of atrial fibrillation, occurring monthly between January 2012 and December 2018, in individuals aged 18 years or over, was extracted from the hospital morbidity database. The number of patients with an atrial fibrillation code documented in this database was used as a proxy for the prevalence of known atrial fibrillation. The number of anticoagulated patients was estimated from total medicine sales of vitamin K antagonists and novel oral anticoagulants (apixaban, dabigatran, edoxaban and rivaroxaban) in mainland Portugal. Descriptive analyses were performed, and seasonal autoregressive integrated moving average (SARIMA) models were built using the R software.

Results: The mean number of episodes of stroke per month was 522 (± 57). The number of anticoagulated patients increased gradually from 68 943 to 180 389 per month. The decreasing trend in the number of episodes has been observed since 2016, along with the increased use of new oral anticoagulants compared to vitamin K antagonists. The final model indicated that the increase in oral anticoagulation use between 2012 and 2018, in mainland Portugal, was associated with a decrease in the number of episodes of stroke associated with atrial fibrillation. It was estimated that the shift in the type of anticoagulation used, between 2016 and 2018, was associated with a reduction of 833 episodes of stroke in patients with atrial fibrillation (4.2%).

Conclusion: The use of oral anticoagulation was associated with a reduced incidence of stroke in patients with atrial fibrillation in mainland Portugal. This reduction was more relevant in the period between 2016 and 2018, and is probably related with the introduction of the novel oral anticoagulants.

Keywords: Anticoagulants/therapeutic use; Atrial Fibrillation/complications; Portugal; Stroke/prevention & control

INTRODUÇÃO

A fibrilhação auricular (FA) é a disritmia persistente mais prevalente na prática clínica,[1] tendo um importante impacto social e económico.[2]

No contexto da morbimortalidade associada à doença, o

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acidente vascular cerebral (AVC) assume particular importância. Estima-se que o risco de AVC – importante causa de morte em Portugal – seja cinco vezes superior nos indivíduos com FA.[3,4]

A anticoagulação oral (ACO), recomendada desde há pelo menos uma década para a redução do risco de AVC na presença de FA, tem visto o seu papel reforçado pela introdução dos novos anticoagulantes orais (NOAC).[5] Atualmente, recomenda-se a introdução de ACO na presença de CHA_2DS_2-VASC superior ou igual a um nos homens e superior ou igual a dois nas mulheres, mediante um perfil de risco hemorrágico aceitável.[1]

Um estudo recentemente publicado, realizado na região norte de Portugal, permitiu estimar que cerca de 73% dos doentes com FA e indicação para anticoagulação se encontravam anticoagulados (dados referentes ao período 2016 - 2018).[6] Este número contrasta com a estimativa prévia de 57% (2015), obtida através do estudo FATA.[7]

Internacionalmente, vários estudos procuraram estimar a relação entre a utilização crescente de ACO e a incidência de AVC, com resultados heterogéneos.[8,9] O estudo de Cowan *et al*, mais recentemente, colmatou algumas das falhas verificadas em estudos anteriores, ao incluir a taxa de prevalência de FA conhecida (em detrimento da taxa de prevalência de FA estimada por estudos de rastreio) e parâmetros relativos à utilização de ACO na população com FA nas suas estimativas para Inglaterra.[10] Neste estudo, verificou-se uma diminuição da taxa de AVC em doentes com FA entre 2011 e 2016, associada à utilização de ACO.[10]

O objetivo principal deste estudo foi avaliar o impacto da utilização de anticoagulantes orais na incidência de AVC associado a FA, em Portugal continental.

MÉTODOS

Para a avaliação do impacto da utilização de anticoagulantes orais na incidência de AVC em doentes com FA, em Portugal continental, consideraram-se os meses entre janeiro de 2012 e dezembro de 2018.

Na impossibilidade de utilizar uma fonte única para a recolha da informação necessária a nível individual, foi necessário adotar uma estratégia multidimensional, com recurso a duas bases de dados reais (*real world data*) para a caracterização das variáveis incluídas na análise: episódios de internamento por AVC em doentes com FA, número de doentes anticoagulados e prevalência de FA. O processo utilizado, para cada uma das variáveis, encontra-se descrito de seguida.

A informação proveio essencialmente de duas fontes: a base de dados de morbilidade hospitalar, cedida ao abrigo de um protocolo de colaboração entre a Administração Central dos Serviços de Saúde, I.P., e o Centro de Estudos de Medicina Baseada na Evidência da Faculdade de Medicina da Universidade de Lisboa, e as estatísticas de vendas, disponibilizadas pela HMR – Health Market Research.

Tratou-se, portanto, de um estudo que não envolveu qualquer experimentação em animais ou humanos, ou quaisquer solicitações junto de participantes. Todos os dados foram disponibilizados de forma irreversivelmente anonimizada e, no caso dos dados de vendas, de forma agregada, sem possibilidade de cruzamento de dados ou identificação dos titulares. O estudo seguiu os princípios da declaração de Helsínquia de 2013 e pelas razões expostas não foi submetido a um pedido de parecer formal por uma comissão de ética.

Episódios de internamento por AVC associado a FA

Para a contabilização dos episódios de internamento relevantes, considerou-se a base de dados de morbilidade hospitalar (BDMH). A BDMH é um registo administrativo de episódios de internamento e episódios selecionados de ambulatório ocorridos em unidades do Serviço Nacional de Saúde (SNS).[11] A BDMH inclui diagnósticos e procedimentos codificados a partir dos processos hospitalares recolhidos por médicos codificadores treinados na utilização da Classificação Internacional das Doenças (*International Classification of Diseases*, ICD).

A identificação dos casos relevantes para análise foi realizada através da ICD na sua nona revisão, modificação clínica (ICD-9-CM) para os anos de 2012 a 2016 e da ICD na sua 10.ª revisão, modificação clínica e sistema de classificação de procedimentos (ICD-10-CM/PCS) nos anos de 2016 a 2018 (a BDMH do ano de 2016 apresenta cerca de 96% dos episódios codificados de acordo com a ICD-9-CM e os restantes 4% de acordo com a ICD-10-CM/PCS). Consideraram-se todos os episódios de internamento, ocorridos durante cada mês do período em análise, em indivíduos com idade superior ou igual a 18 anos, com um diagnóstico principal de AVC (isquémico ou hemorrágico) e um diagnóstico adicional de FA. Os códigos ICD-10-CM/PCS e ICD-9-CM utilizados na análise estão disponíveis no Apêndice 1 (Apêndice 1: https://www.actamedicaportuguesa.com/revista/index.php/amp/article/view/19255/15144).

Número de doentes anticoagulados

Na ausência de informação disponível para o número de doentes com FA anticoagulados, ou para a proporção dos doentes com FA sob ACO, considerou-se o número total de doentes anticoagulados, em Portugal continental, independentemente da indicação terapêutica. A utilização deste parâmetro pressupõe que a evolução nas vendas totais de anticoagulantes (por qualquer indicação) é idêntica à dos anticoagulantes utilizados especificamente na prevenção do AVC nos doentes com FA.

O número de doentes anticoagulados, em cada período

mensal, foi estimado a partir das estatísticas das vendas de antagonistas da vitamina K (acenocumarol e varfarina) e NOAC (apixabano, dabigatrano, edoxabano e rivaroxabano) em Portugal continental, disponibilizadas pela Health Market Research (HMR). Num primeiro passo, calculou-se o total de miligramas vendido, em cada período mensal, para cada um dos fármacos considerados (a partir das características das embalagens: dosagem e número de comprimidos). Num segundo passo, recorreu-se à *defined daily dose* (DDD) para estimar o número de dias de tratamento e, consequentemente, o número de doentes-mês em tratamento.

Prevalência de doentes com FA

Em Portugal, a taxa de prevalência de FA foi estimada pelo estudo FAMA em 2,5% (IC 95%: 2,2% - 2,8%) nos indivíduos de idade superior ou igual a 40 anos.[12] Na população inquirida, todavia, apenas 1,6% tinham conhecimento prévio do diagnóstico. Desde a publicação do estudo FAMA, em 2010, outros estudos concluíram acerca do impacto do envelhecimento da população, bem como da utilização de métodos adicionais de rastreio, na prevalência de FA.[7,13,14] No entanto, que tenhamos conhecimento, não se encontra disponível nenhum estudo longitudinal que caracterize a evolução da taxa prevalência de FA em Portugal e, em particular, da taxa de prevalência de FA conhecida (diagnosticada) ao longo da última década. De notar que a taxa de prevalência de FA conhecida tem particular impacto na análise, na medida em que a introdução da anticoagulação nos doentes com FA implica o diagnóstico prévio desta arritmia.

Como tal, recorreu-se à BDMH para a recolha da informação necessária. Neste caso, estimou-se o número de doentes com idade superior ou igual a 18 anos que, em pelo menos um episódio, receberam a codificação do diagnóstico de FA (em qualquer posição) em cada um dos anos em análise. Os códigos ICD-10-CM/PCS e ICD-9-CM utilizados na análise estão disponíveis no Apêndice 1 (Apêndice 1: https://www.actamedicaportuguesa.com/revista/index.php/amp/article/view/19255/15144). O número de doentes com presença do código de FA na BDMH, assim obtido, foi utilizado como um proxy para prevalência de FA conhecida, refletindo não só o envelhecimento da população, como a possível influência de outros fatores (maior capacidade de diagnóstico e registo) no período em análise. Note-se que, neste caso, optou-se por assumir que o valor anual do parâmetro é válido para cada um dos meses desse ano, anulando o efeito da sazonalidade típica da utilização de cuidados hospitalares.

Análise estatística

Para descrever o número de episódios de AVC ocorridos em doentes com FA em cada instante de tempo *t* foi desenvolvido um modelo de regressão linear com erros SARIMA (*seasonal autoregressive integrated moving average,* modelos autorregressivos integrados de médias móveis sazonais). Este modelo permite descrever o número de episódios de AVC em função de valores registados noutros instantes de tempo, como os valores registados no mês passado ou os valores registados no mesmo mês do ano anterior. Sendo uma generalização da classe dos modelos ARIMA, a classe de modelos SARIMA permite modelar séries com componente sazonal, como os episódios de AVC.

As variáveis foram inicialmente analisadas do ponto de vista descritivo, com recurso a tabelas de frequência e análises de tendência das séries temporais obtidas pelo método de decomposição sazonal de séries temporais por Loess (*seasonal-trend decomposition using Loess*, STL).

Foram depois construídos diversos modelos SARIMA com recurso à função *auto.arima()* do pacote *forecast,*[15] utilizando o *software R* (R Core Team 2019. R*: A language and environment for statistical computing*. R Foundation for Statistical Computing, Vienna, Austria).

Para a construção dos modelos consideraram-se diferentes variáveis explicativas. Para além daquelas já reportadas (número de doentes anticoagulados e número de doentes com FA registados na BDMH), incluíram-se outras séries temporais (proporção de doentes com FA do sexo feminino, idade média dos doentes com FA, severidade dos episódios de AVC nos doentes com FA, o índice de Charlson dos episódios de AVC nos doentes com FA), uma variável categórica referente ao tipo de anticoagulação predominante (antagonistas da vitamina K *versus* NOAC), e um termo de interação entre esta variável categórica e a série do número de doentes anticoagulados.

A seleção do modelo final foi realizada com recurso ao critério de informação de Akaike corrigido (AICc) bem como aos critérios relativos à qualidade preditora [erro quadrático médio (EQM), erro percentual médio (EPM) e erro percentual absoluto médio (EPAM)] e de ajustamento [coeficiente de determinação ajustado (R^2_a)]. A avaliação da qualidade do ajuste aos dados foi realizada através do teste de Ljung-Box utilizando a função *checkresiduals()* do pacote *forecast*.[15] Foram igualmente analisadas as estimativas das funções de autocorrelação (FAC) e de autocorrelação parcial (FACP) amostrais.

RESULTADOS

Análise descritiva

O número de AVC ocorridos em doentes com o diagnóstico de FA variou entre 375 e 661 por mês, sendo em média de 522 (± 57) episódios, com uma mediana de 524 episódios (Fig. 1).

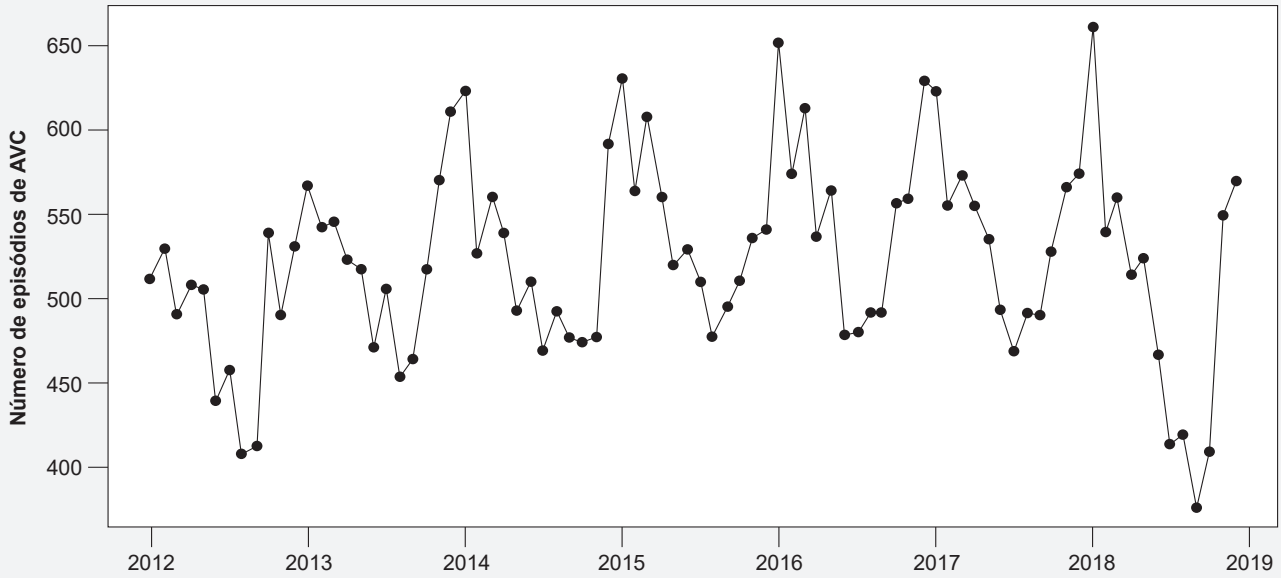

Figura 1 – Cronograma da série do número de episódios de AVC em doentes com FA em Portugal continental, entre janeiro de 2012 e dezembro de 2018
Fonte: Figura elaborada pelos autores a partir dos dados da BMDH 2012 a 2018

A análise pelo método STL permite verificar que se registou um aumento do número de episódios de AVC ocorridos em doentes com FA entre janeiro de 2012 e novembro de 2013 e, novamente, entre julho de 2014 e julho de 2015, e entre janeiro e julho de 2016. Estes períodos são intervalados com períodos de decréscimo do número de episódios. A partir de julho de 2016, e até ao final do período em análise (dezembro 2018), a tendência de decréscimo manteve-se inalterada (Fig. 2).

O número de casos foi superior nos meses mais frios

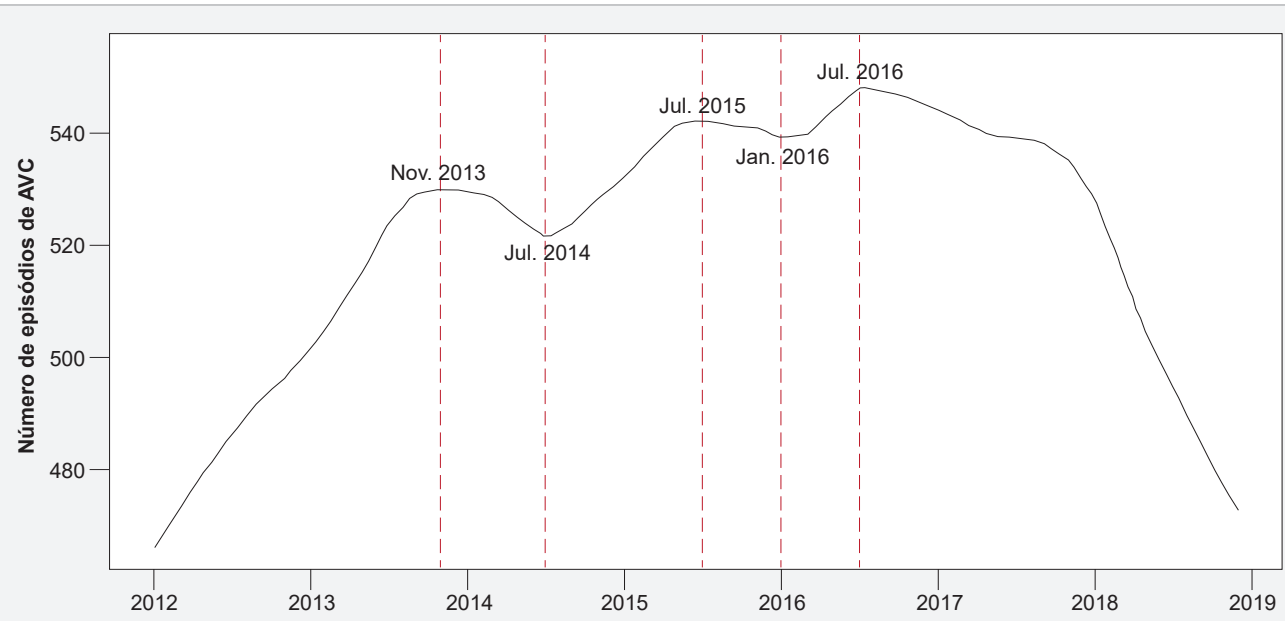

Figura 2 – Tendência da série do número de episódios de AVC em doentes com FA em Portugal continental, entre janeiro de 2012 e dezembro de 2018, obtida pelo método STL
Fonte: Figura elaborada pelos autores a partir dos dados da BMDH 2012 a 2018

(Fig. 3), sugerindo um padrão sazonal, igualmente verificado pelo método STL.

Para a modelização da prevalência de FA conhecida recorreu-se ao número de doentes com registo de FA na BDMH, em cada período anual. Verificou-se uma tendência crescente no período em análise (Fig. 4A).

Quando se procede à padronização do número de episódios de AVC ocorridos em doentes com FA pelo número de doentes com registo de FA (através da divisão do número de episódios de AVC com diagnóstico de FA pelo número de doentes identificados na BDMH, em cada ano), a tendência crescente inicialmente exibida em alguns períodos desaparece, dando lugar a uma tendência globalmente decrescente (Fig. 4B).

Relativamente ao número de doentes anticoagulados, verificou-se um aumento gradual entre janeiro de 2012 e dezembro de 2018 (Fig. 5A), passando de um mínimo de 68 943 para 180 389 indivíduos anticoagulados, por mês. Este aumento deveu-se ao aumento do consumo de NOAC (Fig. 5B). Relativamente ao consumo de NOAC, o aumento foi mais acentuado entre 2014 e 2015.

As Fig.s 2 e 5B sustentam a observação de uma tendência decrescente no número de episódios de AVC em doentes com FA a partir de 2016 e, no início desse mesmo ano, uma alteração na composição da série referente aos ACO (o número de doentes anticoagulados com NOAC suplanta o número de doentes anticoagulados com antagonistas da vitamina K). Esta observação justificou a inclusão de uma variável categórica (tomando valor igual a 1 para as observações nos anos 2016, 2017 e 2018) bem como a adição desta variável categórica em interação com a série do número de doentes anticoagulados.

Modelação do número de episódios de AVC ocorridos em doentes com FA recorrendo a um modelo SARIMA

O modelo com melhor ajuste aos dados para a explicação da evolução do logaritmo dos episódios de AVC ($E_{avc,t}$) (de acordo com os menores valores de AICc, EPM e EPAM e maior valor de R^2_a) utilizou três séries explicativas: o logaritmo do número de doentes tratados com ACO ($\{X_{1,t}\}$), o termo de interação entre a variável categórica (que assumiu valor igual a 1 para as observações nos anos 2016, 2017 e 2018) e o logaritmo do número de doentes tratados com ACO ($\{X_{2,t}\}$) e o número de doentes com codificação de

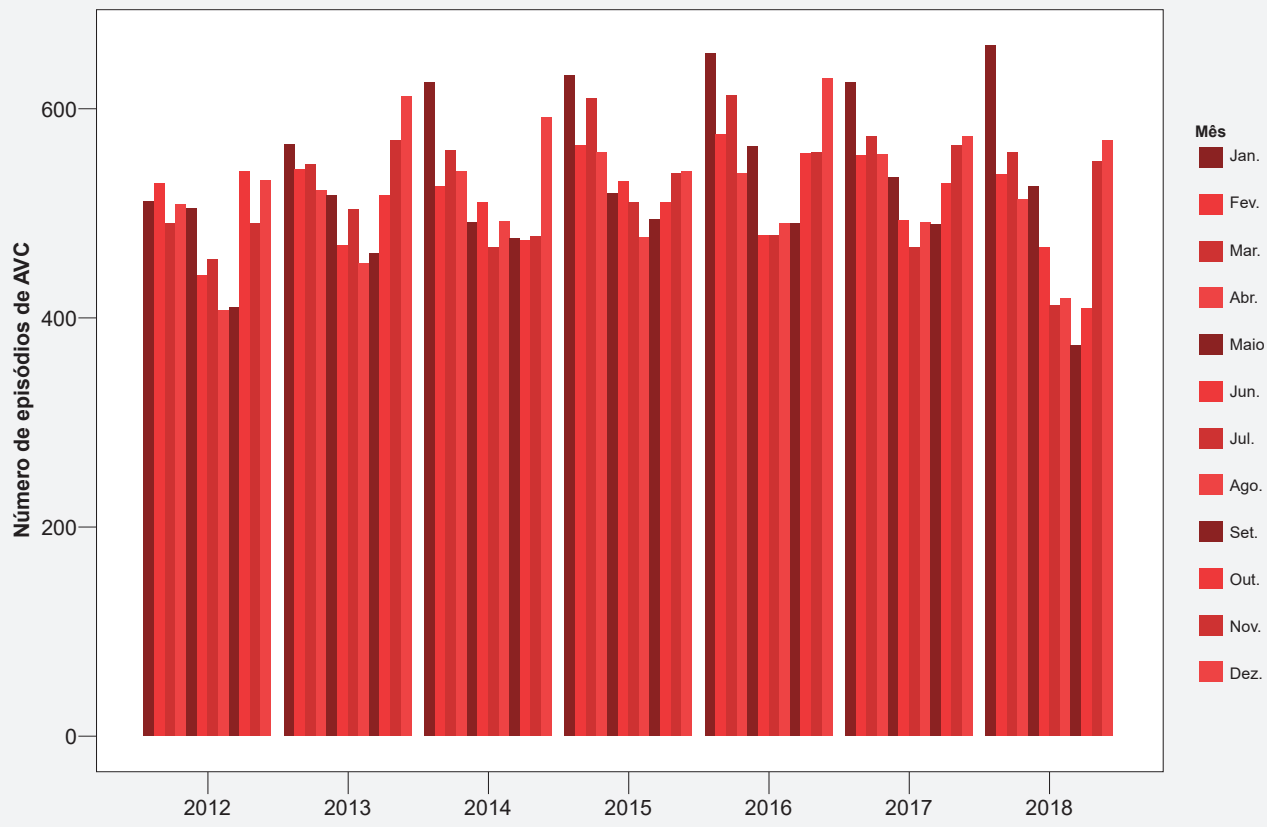

Figura 3 – Gráfico de barras agrupadas, por mês, do número de episódios de AVC em doentes com FA, em Portugal continental, entre janeiro 2012 e dezembro 2018
Fonte: Figura elaborada pelos autores a partir dos dados da BMDH 2012 a 2018

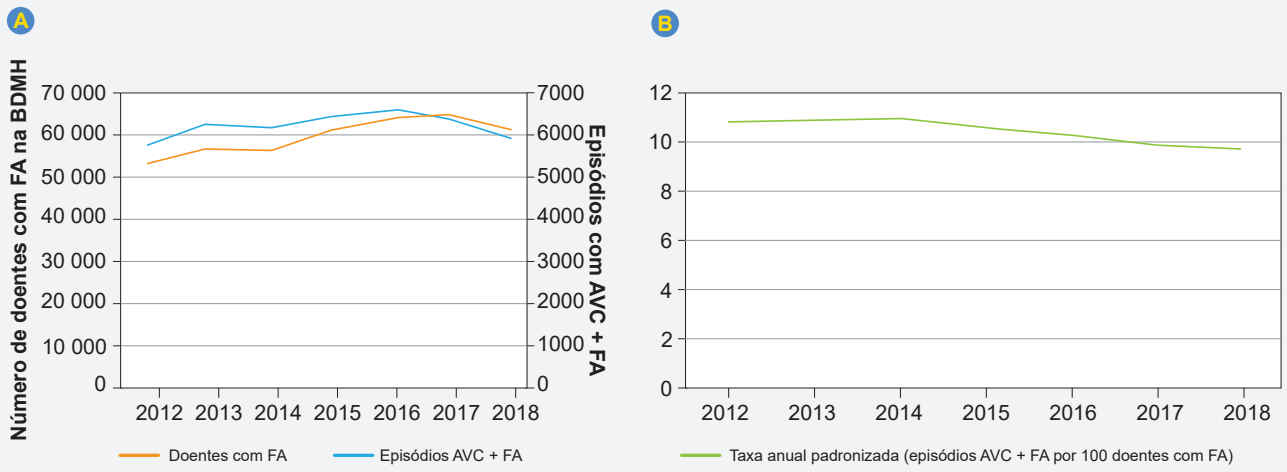

Figura 4 – Taxa anual padronizada de episódios de AVC ocorridos em doentes com FA, em Portugal continental, entre janeiro de 2012 e dezembro de 2018. Séries temporais do nº de doentes com FA e do nº de episódios de AVC + FA BDMH, por ano (A); Taxa anual padronizada de episódios de AVC + FA (episódios por 100 doentes com FA) (B).
Fonte: Figura elaborada pelos autores a partir dos dados da BMDH 2012 a 2018

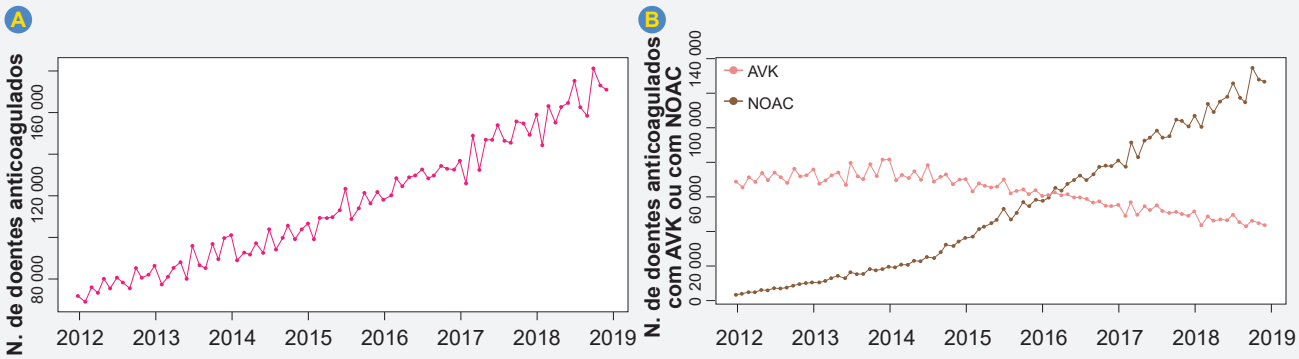

Figura 5 – Cronograma da série do número de doentes anticoagulados, em Portugal continental, entre janeiro de 2012 e dezembro de 2018. Anticoagulantes orais (total) (A); Decomposição em antagonistas da vitamina K (laranja) e anticoagulantes orais não antivitamínicos K (castanho) (B).
Fonte: estimativa dos autores, a partir dos dados de estatísticas de vendas nacionais de fármacos anticoagulantes

FA ($\{X_{3,t}\}$). O valor-*p* do teste Ljung-Box (0,6549) confirma a adequabilidade do modelo para a representação da série temporal em análise, bem como a análise da FAC e FACP amostrais. A equação do modelo é dada por $E_{avc,t} = -0{,}1376\ X_{1,t} - 0{,}0036\ X_{2,t} + 1{,}2137\ X_{3,t} + N_{t-12} + e_t - 0{,}5169\ e_{t-12}$.

Na Fig. 6 apresentamos o cronograma da série que se pretende modelar (cor negra) e o modelo selecionado para ajustar os dados (cor magenta), com intervalo de confiança de 95%, representado a tracejado azul.

O modelo final estimado indica que o aumento do consumo de ACO entre 2012 e 2018, em Portugal continental, foi associado a um decréscimo do número de AVC associados a FA. Os resultados podem ser interpretados da seguinte forma: se o número de doentes anticoagulados aumentar 1% entre janeiro de 2012 e dezembro de 2015 (variável categórica = 0), espera-se que o número de episódios de AVC em doentes com FA decresça 0,1376%. Por outro lado, se o número de doentes anticoagulados aumentar 1% entre janeiro de 2016 e dezembro de 2018 (variável categórica = 1), espera-se que o número de episódios de AVC em doentes com FA decresça 0,1412%. Importa acrescentar que, apesar de este ser o modelo selecionado, todos os modelos identificados e analisados apontavam na mesma direção, ou seja, que a terapêutica com anticoagulantes orais está associada a uma redução no número de episódios de AVC associados a FA.

Este modelo permitiu adicionalmente avaliar um cenário contrafactual para o período entre 2016 e 2018, retirando o efeito sobre a variável resposta determinado pelo *shift* da ACO neste período (incluído através do termo de

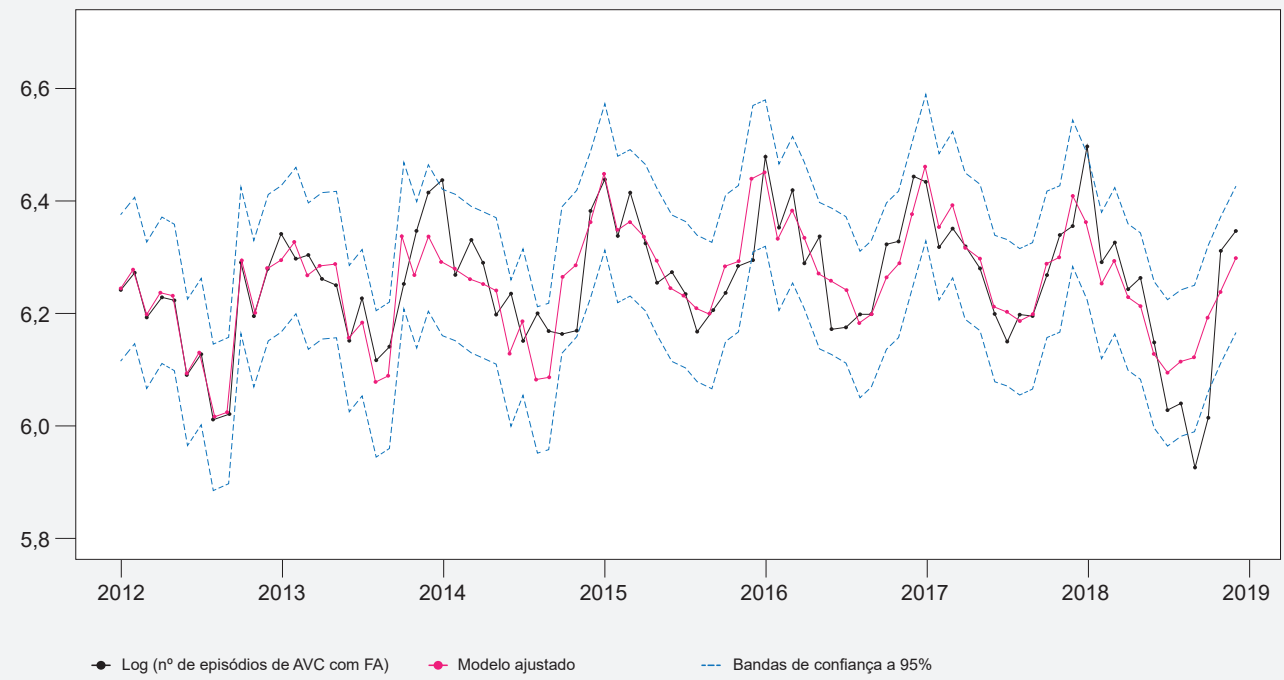

Figura 6 – Cronograma da série do logaritmo do número de episódios de AVC ocorridos em doentes com FA e respetivo modelo ajustado. O modelo ajustado considera três séries explicativas: o logaritmo do número de doentes tratados com ACO, o termo de interação entre a variável categórica (que assumiu valor igual a 1 para as observações nos anos 2016, 2017 e 2018) e o logaritmo do número de doentes tratados com ACO, e o número de doentes com codificação de FA.

interação entre a variável categórica e o número de doentes anticoagulados). Neste caso, a variável categórica assumiria o valor 0 e a equação do modelo para o cenário contrafactual passaria a $E_{avc,t} = -0{,}1376\ X_{1,t} + 1{,}2137\ X_{3,t} + N_{t-12} + e_t - 0{,}5169\ e_{t-12}$.

Neste cenário contrafactual, a estimativa do número de episódios de AVC ocorridos em doentes com FA no período de 2016 a 2018 seria de 19.908 (comparativamente a 19.075 episódios estimados pelo modelo inicial). Desta forma, o efeito do *shift* da ACO entre 2016 e 2018 pode ser quantificado, em termos absolutos, numa redução de 833 episódios de AVC ocorridos em doentes com FA (4,2%).

DISCUSSÃO

O objetivo deste estudo foi avaliar o impacto da utilização de anticoagulantes orais na incidência de AVC associado a FA, em Portugal continental.

Perante a indisponibilidade de uma fonte única para a recolha da informação a nível individual, foi necessário adotar uma estratégia multidimensional, com recurso a dados agregados, obtidos por consulta de duas bases de dados reais (BDMH e estatísticas das vendas de ACO).

Na análise descritiva, verificámos uma tendência maioritariamente crescente do número de episódios de AVC em doentes com FA até 2016, seguida de decréscimo até ao final do período em análise. A análise da taxa anual padronizada de episódios de AVC ocorridos em doentes com FA por 100 doentes com registo de FA na BDMH, pelo contrário, exibiu uma tendência decrescente em todo o período. Por outras palavras, quando anulado o efeito do aumento da prevalência de FA, verifica-se uma tendência decrescente no número de AVC associados a esta arritmia.

O aumento da prevalência de FA, e do AVC associado a FA, nas últimas décadas é reportado por diversos autores, utilizando diversas metodologias para a recolha da informação epidemiológica.[16-19] Entre estes, destacamos a análise de Santos *et al*, por refletir a realidade nacional, utilizando a BDMH como fonte de dados. Nesta análise, reporta-se um aumento de 32% no número de internamentos por AVC entre 2000 e 2014, com um aumento de 138% nos doentes com o diagnóstico secundário de FA.[17] Os autores justificam os resultados, por um lado, com o efeito do envelhecimento da população e maior capacidade de diagnóstico (por exemplo, utilização de meios complementares com maior acuidade diagnóstica, quer em cuidados de saúde secundários quer primários) e por outro, com a melhoria das condições de reporte e codificação de diagnósticos secundários na BDMH.[17] Estes aspetos terão tradução, igualmente, na presente análise. Por esse motivo, parece-nos adequada a utilização do número de doentes com presença

do código de FA na BDMH como um substituto para a prevalência de FA conhecida, refletindo não só o envelhecimento da população, como a possível influência de outros fatores (maior capacidade de diagnóstico e registo).

A análise da série temporal referente aos episódios de AVC em doentes com FA sugere igualmente a presença de sazonalidade, tendência já reportada por outros autores quer para a ocorrência de AVC associado a FA[20] quer para a FA paroxística,[21] sugerindo a influência de fenómenos ambientais (temperatura, humidade) ou de comorbilidades com padrão sazonal (infeções respiratórias e exacerbação de doença pulmonar obstrutiva crónica, por exemplo).

Relativamente ao consumo de ACO, verificou-se um aumento gradual durante todo o período em análise. O aumento mais acentuado, verificado entre 2014 e 2015, no consumo de NOAC justifica-se pela comparticipação pelo SNS (em 2014) dos três primeiros NOAC na prevenção dos eventos tromboembólicos com FA não-valvular (edoxabano juntar-se-ia em 2016). O *shift* na composição relativa do mercado de ACO ocorrido em 2016 (Fig. 5B) justificou a inclusão de um termo de interação entre a variável categórica (que assumiu valor 1 para as observações entre 2016 e 2018) e o número de doentes anticoagulados.

Através da modelação do número de episódios de AVC ocorridos em doentes com FA através de modelo de regressão linear com erros SARIMA, verificámos que o aumento do consumo de ACO entre janeiro de 2012 e dezembro de 2018, em Portugal Continental, foi associado a um decréscimo do número de AVC ocorridos em doentes com FA.

A diminuição na incidência de AVC associado a FA está em linha com o verificado por outros autores, utilizando dados de vida real, internacionalmente. Em Estocolmo (Suécia), num estudo observacional retrospetivo incluindo doentes com FA, verificou-se o aumento da utilização de ACO (de 51,6% para 73,8%) e a diminuição da incidência de AVC isquémico (de 2,01 para 1,17 por 100 pessoas-ano) entre 2012 e 2017.[18] Através de um modelo de Poisson, a anticoagulação oral foi associada a redução de 10% no risco absoluto de AVC isquémico [*incidence rate ratio* (IRR) de 0.63 (IC 95%: 0,58 - 0,69) antes e 0.73 (IC 95%: 0,66 - 0,80) após ajuste pela utilização de ACO].[18] Em Inglaterra, também através de um modelo Poisson (incluindo o ajuste para a prevalência de FA) o aumento de 1% na utilização de ACO foi associado a uma diminuição de 0,8% na taxa semanal de AVC associado a FA [IRR 0,992 (IC 95%: 0,989 - 0,994)].[10]

A viabilização da análise foi conseguida por meio de algumas concessões e pressupostos, o que acarretou limitações. À semelhança de outros autores,[10] utilizámos dados agregados. Este facto poderá ter acarretado vieses para a análise, cujo impacto não pôde ser mensurado. Por exemplo, não é possível confirmar se o diagnóstico de FA ou o início da anticoagulação era prévio ao episódio de internamento por AVC. Da mesma forma, não é possível assegurar que a causa dos episódios de AVC em doentes com o diagnóstico de FA (AVC associado a FA) seja a FA.

Este estudo não pretendeu estimar a prevalência da FA em Portugal nem a incidência de AVC. De facto, a metodologia utilizada (baseada na identificação dos casos e eventos na BDMH) não inclui todos os doentes com FA, nomeadamente, os doentes com FA que não recorreram a cuidados hospitalares, nem todos os episódios de AVC, nomeadamente, alguns casos sem internamento e aqueles que ocorrem no sector privado. Apesar de os valores apresentados não corresponderem, assim, a estimativas absolutas robustas de prevalência de FA e incidência de AVC, tal não é crítico dado que o que se pretendia era a avaliação da variação ao longo do tempo.

Por outro lado, não nos foi possível integrar outras variáveis, inclusivamente de forma agregada, como seja o risco tromboembólico ou hemorrágico de base. Outra limitação foi pressupor que a evolução do número total de doentes anticoagulados no período de 2012 a 2018 reflete o que terá sucedido, especificamente, nos doentes com FA. Apesar de relevante, este pressuposto parece-nos aceitável, na medida em que as restantes indicações para ACO se referem, regra geral, a uma utilização restrita no tempo (ao contrário do que sucede com a FA). Por último, não foi possível estender a análise aos anos de 2019 e seguintes, dada a indisponibilidade dos dados da BDMH para este período.

CONCLUSÃO

A utilização de anticoagulantes orais associou-se à redução da incidência de AVC em doentes com FA, em Portugal Continental. Esta redução foi mais relevante no período entre 2016 e 2018, em provável relação com o aumento do consumo de ACO, resultante da introdução dos NOAC.

AGRADECIMENTOS

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CONTRIBUTO DOS AUTORES

RA: Redação do primeiro rascunho do manuscrito.

MG, FL: Análise dos dados.

Todos os autores contribuíram para a conceção e desenho do estudo, interpretação dos resultados e revisão crítica, e todos leram e aprovaram a versão submetida.

PROTEÇÃO DE PESSOAS E ANIMAIS

Os autores declaram que os procedimentos seguidos estavam de acordo com os regulamentos estabelecidos pelos

responsáveis da Comissão de Investigação Clínica e Ética e de acordo com a Declaração de Helsínquia da Associação Médica Mundial atualizada em 2013.

CONFIDENCIALIDADE DOS DADOS

Os autores declaram ter seguido os protocolos do seu centro de trabalho acerca da publicação de dados.

CONFLITOS DE INTERESSE

RA, FL, MB, LSM e JC eram membros, à data, do Centro de Estudos de Medicina Baseada na Evidência (CEMBE) da Faculdade de Medicina da Universidade de Lisboa. Este centro de investigação dedica-se à educação pré e pós-graduada e, desde 2002, realizou diversos projetos de investigação clínica, epidemiológica e farmacoeconómica, que receberam *unrestricted grants* de mais de 20 empresas farmacêuticas, incluindo AstraZeneca, Bayer, Boehringer Ingelheim, Daiichi Sankyo e Sanofi.

MG e RO tiveram acesso aos dados necessários para a realização do estudo através do Centro de Estudos de Medicina Baseada na Evidência (CEMBE).

DC recebeu pagamentos ou honorários por palestras, apresentações, gabinetes de oradores, redação de manuscritos ou eventos educacionais; recebeu apoio para atividades educacionais de Daichi Sankyo, Ferrer, BIAL; participou em reuniões educacionais e/ou participou em conferências ou simpósios (tendo recebido apoio para viagens, alojamento e/ou hospitalidade) organizadas por Bial, Bristol-Myers Squibb, Bayer, Boehringer Ingelheim, Daiichi Sankyo, Merck Serono, Ferrer, Pfizer, Novartis e Roche.

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Prevalence Rate of Thalassemia Carriers among Individuals with Microcytosis or Hypochromia in Portugal

Prevalência de Portadores de Talassémia em Indivíduos com Microcitose ou Hipocromia em Portugal

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ABSTRACT

Introduction: Microcytosis and hypochromia result from deficient hemoglobin synthesis in red blood cells and are easily detected in a complete blood count test. These conditions are mainly due to iron nutritional deficiency, but may also result from some genetic diseases, such as thalassemia. The aim of this study was to determine the contribution of β- and α-thalassemia to these abnormal hematological phenotypes in a representative sample of adult individuals living in Portugal who participated in the first Portuguese National Health Examination Survey (INSEF).

Methods: Among the 4808 INSEF participants, 204 had microcytosis, hypochromia or both. The corresponding 204 DNAs were screened for changes in the β-globin gene by next-generation sequencing and Sanger sequencing. In addition, α-thalassemia deletions within the α-globin cluster were investigated by Gap-PCR and multiplex ligation-dependent probe amplification.

Results: In this selected subgroup of INSEF participants, 54 had α-thalassemia (26%), predominantly caused by the -$\alpha^{3.7kb}$ deletion, and 22 were β-thalassemia carriers (11%) mainly due to point mutations in the β-globin gene previously known in Portugal.

Conclusion: Thalassemia trait is a frequent cause of microcytosis or hypochromia in Portugal since this genetic condition was found in 37% of the investigated cases.

Keywords: Erythrocytes; Erythrocyte Indices; Hematologic Tests; Portugal; Thalassemia/diagnosis; Thalassemia/genetics

RESUMO

Introdução: A microcitose e a hipocromia são alterações nos glóbulos vermelhos resultantes de um défice de síntese da hemoglobina e são facilmente identificáveis aquando da realização de um hemograma. Estas condições são, em grande maioria, devidas a um défice nutricional em ferro, contudo podem ser consequência de algumas doenças genéticas, como por exemplo a talassémia. Neste trabalho, pretendemos determinar a contribuição da β- e da α-talassémia para a ocorrência destes fenótipos hematológicos anómalos, numa amostragem representativa de indivíduos adultos residentes em Portugal e que participaram no primeiro Inquérito Nacional de Saúde com Exame Físico (INSEF).

Métodos: De entre os 4808 participantes no estudo INSEF, 204 apresentavam microcitose, hipocromia ou ambas. Os 204 ADNs correspondentes a estes indivíduos foram usados para pesquisa de alterações no gene da β-globina por sequenciação de nova geração e por sequenciação de Sanger. Para além disso, foram pesquisadas deleções α-talassémicas no agrupamento génico da α-globina por Gap-PCR e *multiplex ligation-dependent probe amplification*.

Resultados: Neste subgrupo selecionado de participantes no estudo INSEF, 54 tinham α-talassémia (26%), predominantemente devida à deleção -$\alpha^{3.7kb}$, e 22 eram portadores de β-talassémia (11%) devido à presença de mutações pontuais no gene da β-globina na sua grande maioria já anteriormente observadas em Portugal.

Conclusão: Este estudo revelou que o traço talassémico é uma causa frequente de microcitose ou hipocromia em Portugal, uma vez que foi detetado em 37% dos casos investigados.

Palavras-chave: Eritrócitos; Índices de Eritrócitos; Portugal; Talassémia/diagnóstico; Talassémia/genética; Testes Hematológicos

INTRODUCTION

Microcytosis and hypochromia result from deficient hemoglobin synthesis in precursors of erythroid cells, causing a reduction in both mean corpuscular volume and mean corpuscular hemoglobin of mature red blood cells, which are easily detected when performing a complete blood count in a hematology analyzer. These changes may be associated with anemia and are a consequence of genetic or acquired conditions, such as iron deficiency, α-thalassemia or β-thalassemia traits. The abnormal morphological findings do not allow, by themselves, to differentiate the possible causes. Therefore, in order to find their origin, a battery of diagnostic tests is required, including measurement of serum ferritin, transferrin saturation or total iron binding capacity (to diagnose iron deficiency), hemoglobin A2 level estimation or screening for mutations in the β-globin gene (to diagnose β-thalassemia trait), and screening for molecular lesions in the α-globin genes by molecular techniques (to diagnose α-thalassemia).

Iron deficiency is the leading cause of the microcytic hypochromic anemia worldwide.[1] In developed countries,

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iron deficiency is frequently caused by insufficient dietary iron intake or by conditions that cause either iron loss or decreased iron absorption.[2] In our country, a recent study that analysed adults aged over 18 years old living in mainland Portugal estimated a prevalence rate of iron deficiency anemia of 10.9% when considering participants with both anemia and ferritin below 30 ng/mL, and a prevalence rate of iron deficiency of 31.9% when considering participants without anemia but with ferritin below 30 ng/mL.[3]

The hemoglobinopathies encompass all genetic diseases of hemoglobin. They fall into two main groups: thalassemias and structural hemoglobin variants. Thalassemias are among the most common human genetic diseases worldwide and are caused by reduced production of the α- or β-globin chains of hemoglobin, resulting in α- or β-thalassemia, respectively.[4] The worldwide distribution of α-thalassemia is quite similar to that of β-thalassemia, extending from sub-Saharan Africa, throughout the Mediterranean region and Middle East, to the Indian sub-continent and East and Southeast Asia. Through recent migration and mobility flows, thalassemias are widely prevalent across the world, including the American continent, Australia, Western Europe and, in more recent years, Northern Europe.[5,6]

The prevalence rate of hemoglobinopathies in Portugal was estimated by epidemiological studies in the nineties. The α-thalassemia trait was observed in 10% of the newborns.[7] Moreover, a countrywide adult male based study revealed a prevalence rate of β-thalassemia trait of 0.45% in mainland Portugal.[8] However, an uneven distribution throughout the country with some regional prevalence rates as high as 5% were observed.[8-10] These results supported the implementation of a Nationwide program (Programa Nacional de Controlo das Hemoglobinopatias - PNCH) as well as the publication of a national guideline on hemoglobinopathies by the Directorate General of Health (Direção-Geral da Saúde) in 2004.[11] As far as we know, no large epidemiological studies about this subject and no monitoring of the application of the guideline have been conducted in Portugal since then.

In this study, we aimed to estimate the contribution of α- and β-thalassemia to the presence of microcytosis or hypochromia phenotypes in a representative sample of adult individuals living in mainland Portugal, and in the autonomous regions of Madeira and Azores, who participated in the first Portuguese National Health Examination Survey (INSEF).[12] In addition, we intended to identify the molecular basis underlying the α- and β-thalassemia cases and to evaluate their corresponding hematological phenotypes. Moreover, other aims of this study were to analyse the demographic characteristics of thalassemia carriers in order to assess if they were followed-up by a general practitioner/family physician and to understand their self-perception about their health status.

METHODS

Study design and population

This is a sub-study of the first Portuguese National Health Examination Survey (INSEF),[12] a cross-sectional population-based survey previously performed by INSA in 2015. The survey included three components: physical examination, blood collection and health interview, targeting non-institutionalized individuals, aged between 25 and 74 years old, living in Portugal for more than 12 months, as described elsewhere.[12] The INSEF sample was selected using a two-stage probabilistic cluster design, stratified by region and degree of urbanization (rural/urban). Of the total selected individuals, 4911 effectively participated. However, the existence of a chronic disease preventing blood collection, or a known severe anemia were considered exclusion criteria for blood collection. In addition, some cases were excluded due to unavailable data regarding the subsequent blood tests or unsuccessful DNA extraction. Therefore, in the current study, the sample was restricted to 4808 participants.

Blood samples and data collection

After obtaining the participants' written informed consent, trained healthcare professionals conducted all the INSEF procedures in primary health care centers. The information regarding the demographic and health status of participants was collected using a computer-assisted personal interview software. For this study, the following variables were considered: age, sex, nationality, region of residence and degree of urbanization (rural/urban). Self-perception of health status was assessed with the question "In general, how do you rate your health? (very good/good/fair/poor/very poor)". Participants were also inquired if they were followed by a family physician (yes/no). In addition, participants were asked if they were aware of having anemia, diagnosed by a medical doctor, (yes/no). Pregnancy and tobacco smoking status were also self-reported.

Venous non-fasting blood samples were collected in EDTA Vacutainer® tubes to perform a complete blood count (CBC) in each regional laboratory, as previously described.[12] CBC includes the measurement of the following hematological parameters: red blood cells (RBC), hemoglobin (Hb), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), hematocrit (Ht), and red cell distribution width (RDW). Anemia was defined as Hb < 12.0 g/dL for female, < 11.0 g/dL for pregnant female, and < 13.0 g/dL for male subjects.[13] Hemoglobin levels were adjusted for smoking according to World Health Organization (WHO) recommendations.[13] No adjustment was performed for altitude, since

all collection sites were under 1000 m. Microcytosis was defined when MCV < 80 fL and hypochromia when MCH < 27 pg.[14]

INSEF samples were stored in the central facilities of INSA and were used for DNA extraction in a nucleic acid extractor, MagNA pure LC (Roche®, Germany). DNA quantity and quality were assessed using a spectrophotometer NanoDrop One (Thermo Fisher Scientific®, USA).

Beta-globin gene screening for mutations by next-generation sequencing (NGS)

In order to screen for mutations in the β-globin gene (*HBB*), a DNA fragment of 2106 bp was amplified, from c.-159 (in 5'UTR) to c.*474 (in 3'UTR), using the forward primer 5'- TAAGCCAGTGCCAGAAGAG -3' and the reverse primer 5'- GAGTCAAGGCTGAGAGATGCAGGA -3' in a T-Gradient Thermocycler, Biometra®. Amplicon purification was performed using the Agent AMPure XP PCR Purification kit (Beckman Coulter®, USA) followed by quantification in a Qubit 3.0 fluorometer (Life Technologies®, USA). To prepare the sequencing libraries, the Nextera XT DNA Library Prep (Illumina®, USA) was used. Libraries were sequenced in a MiSeq equipment (Illumina®, USA) using a 0.3 Gb flow cell. Data analyses of the sequencing results comprised three steps: quality control using the MultiQC® 1.6.dev0 software; mapping reads to reference genome GRCh38 using bowtie; and variant calling where base call quality values were corrected for systematic error with the software GATK®. Varying positions were filtered for variant quality and genotypes were only considered when there was a minimum read depth of 20x. Genotypes with an allelic balance below 30% and above 70% were excluded.

Beta-globin genetic variants validation by Sanger sequencing

Validation of each type of genetic variant found by Next-generation Sequencing (NGS) was done by Sanger sequencing after PCR purification using ExoSAP-IT PCR Product Clean-up (Applied Biosystems®). Sanger sequencing was performed using the ABI Prism Big Dye Terminator v1.1 Cycle Sequencing kit (Applied Biosystems®) in a 3500 Genetic Analyzer (Applied Biosystems®). Sequences were analysed using the software FinchTV v1.4.0 (Geospiza®). The obtained sequences were compared with the reference sequences, using Ensembl[15]: ENSG00000244734 for *HBB* gene sequence; ENST00000335295.4 for the corresponding transcript and the UniProtKB P68871 for the β-globin protein sequence.

Deletional alpha-thalassemia screening

The common -$\alpha^{3.7kb}$ deletion was detected by Gap-PCR as described.[16] DNA samples negative for this deletion were screened for copy number variation in the α-globin gene cluster by multiplex ligation-dependent probe amplification (MLPA) using the SALSA MLPA® probemix P140-C1-0415 HBA (MRC-Holland). The amplified fragments were isolated by capillary electrophoresis in a 3130xl genetic analyzer, ABI PRISM® (Applied Biosystems®) and evaluated using the Coffalyser.net software (MRC-Holland).

Statistical analyses

The statistical analyses were performed using the SPSS® software (IBM® Corp. Released 2018. IBM® SPSS Statistics for Windows®, Version 26.0. Armonk, NY: IBM® Corp). For descriptive analysis, continuous variables were represented as mean and standard deviation, median, minimum, and maximum values; for categorical variables, absolute and relative frequencies were used.

The genotype/phenotype association study was performed comparing the hematological parameters (RBC, Hb, MCV, MCHC, MCH, Ht, and RDW) between two subgroups of the selected participants. One group included the "Thalassemia carriers", and the other group was composed of participants without thalassemia but with microcytosis, hypochromia, or both. Due to sex-related differences, RBC and Hb comparisons were stratified by sex. Group comparisons were performed using the parametric T-test. To evaluate the normality assumption within groups, the Shapiro-Wilk normality test was applied. To test the homogeneity of variance assumption we used Leven's test. For variables that did not follow the normal distribution, the Mann-Whitney test was used for comparisons. Statistical significance was defined as p-value < 0.05.

RESULTS

Hematological phenotype of the selected participants and their demographic characteristics

The overall sample included in this study comprised 4808 individuals, 2573 (53.5%) female and 2235 (46.5%) male (ratio female/male of 1.2). Following blood tests, there was microcytosis, hypochromia, or both in 204 (4.2%) participants (Table 1), who were selected for β- and α-thalassemia molecular screening. Within this selected group, 157 were female (77%) and 47 male (23%), presenting a mean age of 49.3 ± 11.8 years old, and a ratio female/male of 3.3. Hypochromia was observed in 201 individuals (98.5%), while microcytosis was observed in 112 individuals (54.9%). The two phenotypes were detected simultaneously in 109 individuals (53.4%). Regarding the Hb level, 88 out of the 204 individuals (43.1%) had anemia, which was observed in 14 of the 47 male (29.8%) and in 74 of the 157 female subjects (47.1%).

Most of the 204 selected participants were Portuguese (n = 172; 84.3%), and 32 (15.7%) had another nationality:

Table 1 – Hematological parameters of the selected 204 individuals presenting microcytosis or hypochromia in the first Portuguese National Health Examination Survey

Hematological parameters	Mean	SD	Median	Min.	Max.
RBC (x10^{12}/L)_Male	5.67	0.08	5.71	4.30	6.70
RBC (x10^{12}/L)_Female	4.78	0.04	4.77	3.75	7.30
Hemoglobin (g/dL)_Male	13.9	0.3	14.2	9.8	16.7
Hemoglobin (g/dL)_Female	11.9	0.1	12.0	6.5	15.3
MCV (fL)	77.7	0.4	79.5	56.9	86.9
MCH (pg)	24.8	0.2	25.6	16.2	28.1
MCHC (g/dL)	31.9	0.1	31.9	28.3	36.1
Hematocrit (%)	38.6	0.3	38.1	23.0	52.0
RDW (%)	15.4	0.1	15.2	12.1	22.3

SD: standard deviation; Min.: minimum; Max.: maximum; RBC: red blood cells; MCV: mean corpuscular volume; MCH: mean corpuscular hemoglobin; MCHC: mean corpuscular hemoglobin concentration; RDW: red cell distribution width

Angola, Brazil, Cape Verde, France, South Africa, Bulgaria, Canada, China, India, Mozambique, Moldova, Pakistan, and Syria.

The 204 selected participants with microcytosis or hypochromia were found distributed by all the seven Regional Health Administration (ARS) areas: in mainland Portugal - North (n = 27), Centre (n = 17), Lisbon and Tagus Valley (n = 29), Alentejo (n = 35), and Algarve (n = 35), and in the autonomous regions of Madeira (n = 28) and Azores (n = 33). Forty-nine (24%) were rural residents and 155 (76%) were living in urban areas.

Alpha-thalassemia diagnosis

The results regarding α-thalassemia, obtained by Gap-PCR and MLPA methodologies,[16-18] are shown in Table 2. Of the 204 participants, 54 had α-thalassemia (26.5%). The 3.7kb deletion was found in 52 participants with the heterozygous genotype (-α$^{3.7kb}$/αα) and in one with the homozygous genotype (-α$^{3.7kb}$/-α$^{3.7kb}$). The less common α-thalassemia deletion of 4.2kb was found in one participant (-α$^{4.2kb}$/αα). Another change in the *HBA* cluster was found in two participants: the triple α-globin gene (ααα$^{anti-3.7kb}$/αα).

Beta-thalassemia diagnosis

The screening for changes in the *HBB* gene, performed by NGS, revealed 29 different genetic variants. However, 19 of them were considered benign, probably not affecting gene expression because they were located outside of the gene-coding regions or occurred within introns (12 were deep-intronic and 7 were located in the 3'UTR). Therefore, these genetic variants will not be presented in this study because they are unlikely to have clinical relevance. The other 10 different genetic variants, which were classified as pathogenic or probably pathogenic by ClinVar, were validated by Sanger sequencing, and are shown in Table 3. Twenty-two participants (10.8%) were classified as β-thalassemia carriers, and other three were detected as carriers of a hemoglobin structural variant (Hb S, Hb C, and Hb D-Portugal).

The four more common β-thalassemia point mutations detected in this study were the same already described as prevalent in Portugal: codon 39 (C>T), IVS-I-1 (G>A), IVS-I-6 (T>C) and IVS-I-110 (G>A).[19] The less common mutations, codon 15 (G>A) and codon 6 (-A), have already been described as occurring less frequently in Portugal.[19] The β-thalassemia deletion, codon 41/42 (-CTTT), was detected in this study for the first time in Portugal, but was found in an individual born in a foreign country.

One β-thalassemia carrier (mutation IVS-I-6 T>C) was also diagnosed with α-thalassemia (-α$^{3.7kb}$/αα), and consequently is considered double heterozygous. The same was observed for the HbS carrier, who also has the

Table 2 – Pathogenic deletions and insertions affecting the alpha-globin genes in the 204 studied individuals with microcytosis or hypochromia

Molecular lesion	Genotypes	Consequences	Number of cases	
			(n)	(%)
3.7 kb deletion	**–α$^{3.7kb}$/αα**	α-thalassemia	52	25.5
3.7 kb deletion	**–α$^{3.7kb}$/–α$^{3.7kb}$**	α-thalassemia	1	0.5
4.2 kb deletion	**–α$^{4.2kb}$/αα**	α-thalassemia	1	0.5
3.7 kb insertion	**αα/ααα$^{anti3.7kb}$**	-	2	0.9
none	**αα/αα**	-	148	72.6

Table 3 – Pathogenic changes detected in the beta-globin gene in the 204 studied individuals with microcytosis or hypochromia

	Genomic position*	rs identification	HGVS nomenclature**	Common nomenclature	Consequence	Number of cases (n)	Number of cases (%)
Pathogenic variants detected in *HBB* gene	g.5226774	rs11549407	c.118C>T	Cd39 (C>T)	β-thalassemia	8	3.9
	g.5226924	rs35724775	c.92+6T>C	IVS-I-6 (T>C)	β-thalassemia	4	1.9
	g.5226929	rs33971440	c.92+1G>A	IVS-I-1 (G>A)	β-thalassemia	3	1.5
	g.5226820	rs35004220	c.93-21G>A	IVS-I-110 (G>A)	β-thalassemia	3	1.5
	g.5226974	rs34716011	c.48G>A	Cd15 (G>A)	β-thalassemia	2	0.9
	g.5227001	rs63749819	c.20del	Cd6 (-A)	β-thalassemia	1	0.5
	g.5226762	rs80356821	c.126_129del	Cd41/42 (-CTTT)	β-thalassemia	1	0.5
	g.5227002	rs334	c.20A>T	Hb S	Hemoglobin variant	1	0.5
	g.5227003	rs33930165	c.19G>A	Hb C	Hemoglobin variant	1	0.5
	g.5225678	rs33946267	c.364G>C	Hb D-Portugal	Hemoglobin variant	1	0.5
Without alterations in *HBB* gene						179	87.5

*: genomic coordinates according to (GRCh38.p12); **: reference sequence based on a protein coding mRNA (NM_000518.5)

α-thalassemia allele (-$\alpha^{3.7kb}$/αα).

Association of thalassemia with the hematological phenotype

After the molecular characterization, the population presenting microcytosis or hypochromia was divided in two groups: the group of "Thalassemia carriers" (n = 75), which includes the α-thalassemia carriers, the β-thalassemia carriers, the β- and α-double heterozygous, and the homozygous for the -$\alpha^{3.7kb}$ allele; and the group of "Individuals without thalassemia" (n = 129). The comparison of the hematological parameters between these two groups is presented in Table 4. The group of thalassemia carriers had a higher number of RBCs and a higher level of Hb in female subjects ($p < 0.001$, and $p = 0.007$, respectively). No differences in male subjects were observed for these hematological parameters. In addition, thalassemia carriers had a higher Ht ($p < 0.001$) and a lower RDW ($p < 0.001$) than the group without thalassemia.

If we compare only the β-thalassemia carriers (n = 22) with the group without thalassemia, they had higher values of RBC [mean 6.08 ± 0.41 x10^{12}/L for males ($p = 0.013$) and 5.64 ± 0.54 x10^{12}/L for females ($p < 0.001$)], and a marked microcytosis [MCV, mean 66.3 ± 6.0 fL ($p < 0.001$)] and hypochromia [MCH, mean 20.8 ± 2.1 pg ($p < 0.001$)]. Moreover, more than half of the β-thalassemia carriers (54.5%) also presented with anemia.

On the other hand, if we compare the α-thalassemia carriers (n = 54) with the group without thalassemia, they were characterized by mild microcytosis or normocytosis [MCV, mean 81.2 ± 3.2 fL ($p < 0.001$)] but all of them had hypochromia [MCH, mean 25.9 ± 1.0 pg ($p = 0.005$)] and only nine (17%) had anemia.

The only individual (a male) with a double heterozygosity for β- and α-thalassemia, revealed a very mild phenotype, with no anemia or microcytosis (Hb = 14.5 g/dL, MCV = 82.3 fL). He only presented with hypochromia (HGM = 24.4 pg).

Demographic and health characteristics of the thalassemia carriers

The group positive for thalassemia (n = 75) is comprised of 44 females and 31 males. They come from all the aforementioned seven regions of Portugal. Eighteen are rural residents and 57 are living in urban areas. Almost all β-thalassemia carriers had Portuguese nationality (99%). On the other hand, a wider range of nationalities was found in α-thalassemia carriers: 36 from Portugal and 17 from Brazil, Cape Verde, Angola, France, India, and Pakistan.

Although 88 out of the 204 selected participants had anemia, according to their self-reported health, anemia was only self-reported by four female participants, one of them having a β-thalassemia trait. There was no male self-reported anemia. One woman with α-thalassemia self-reported pregnancy but did not report anemia, which turned out to be true.

Most thalassemia carriers reported their health as fair (n = 44, 58.7%) or good (n = 25, 33.3%). Most thalassemia carriers had a family physician (n = 56, 74.7%). Considering only the β-thalassemia carriers, 15/22 (68%) had a family physician.

Table 4 – Hematological parameters in the studied population (n = 204 from the first National Health Examination Survey) with microcytosis or hypochromia – comparison between two subgroups. "Thalassemia carriers" *versus* other "Without thalassemia".

Hematological parameters	Individuals without thalassemia (n = 129)					Thalassemia carriers* (n = 75)					*p*-value
	Mean	SD	Median	Min.	Max.	Mean	SD	Median	Min.	Max.	
RBC ($x10^{12}$/L)_Male	5.45	0.62	5.38	4.45	6.70	5.77	0.53	5.80	4.30	6.60	0.073[a]
RBC ($x10^{12}$/L)_Female	4.67	0.46	4.70	3.75	7.30	5.08	0.52	5.08	4.29	6.40	**< 0.001**[b]
Hemoglobin (g/dL)_ Male	13.4	2.32	14.0	9.8	16.7	13.2	1.3	13.3	11.4	14.8	0.291[b]
Hemoglobin (g/dL)_ Female	11.7	1.3	11.8	6.5	15.3	12.2	1.1	12.4	9.0	14.8	**0.007**[b]
MCV (fL)	78.3	5.2	78.8	57.2	85.9	76.8	8.0	80.9	56.9	86.9	0.969[b]
MCH (pg)	25.0	2.0	25.6	16.2	28.1	24.4	2.7	27.7	17.2	26.9	0.521[b]
MCHC (g/dL)	31.9	1.1	31.9	28.3	36.1	31.8	0.9	31.8	28.7	33.5	0.603[b]
Hematocrit (%)	37.2	4.4	37.0	23.0	52.0	41.0	4.4	40.7	31.4	51.2	**< 0.001**[a]
RDW (%)	15.8	1.9	15.6	12.3	22.3	14.7	1.5	14.5	12.1	19.1	**< 0.001**[b]

*: The group named "Thalassemia Carriers" includes 73 beta-thalassemia or alpha-thalassemia heterozygous, one homozygous for the 3.7 kb alpha-thalassemia deletion, and one double heterozygous for beta-thalassemia and alpha-thalassemia alleles. SD: standard deviation; Min.: minimum; Max.: maximum; RBC: red blood cells; MCV: mean corpuscular volume; MCH: mean corpuscular hemoglobin; MCHC: mean corpuscular hemoglobin concentration; RDW: red cell distribution width. **a**: T-test; **b**: Mann-Witney test; significant *p*-value < 0.05 are shown in bold.

DISCUSSION

Although iron deficiency and iron deficiency anemia are, in fact, the most likely cause for the presence of an abnormal hematological phenotype of microcytosis or hypochromia,[2,3,20] our study reinforces the role played by thalassemia as a possible cause for that. Our results suggest that 26.5% of those cases are due to α-thalassemia and 10.8% are due to β-thalassemia, which is a considerable high prevalence rate in both cases. The differentiation of thalassemia trait from iron deficiency and iron deficiency anemia is important for two main reasons. First, misdiagnosed β-thalassemia carriers will be deprived of genetic counselling and may be at risk of having seriously affected offspring. Moreover, in this case, if unnecessary iron is prescribed, individuals will not improve their hematological phenotype and it could even lead to excessive accumulation of iron deposits in the tissues, known as hemosiderosis,[21] in addition to having unnecessary expenses concerning medicines.

We observed a female/male ratio of 1.2 in general participants of INSEF, but after applying the selection criteria of "to have microcytosis or hypochromia" this ratio increased to 3.3, with a great predominance of affected female individuals. One reason for this may be because iron deficiency is more prevalent in women than in men.[3,20] Nevertheless, after the diagnosis of thalassemia, the ratio of female/male returned to 1.4 in these carriers, which is similar to the original ratio in INSEF participants. This is because thalassemia is a group of autosomal genetic diseases due to affected globin genes, which are located in chromosomes 11 and 16. Consequently, thalassemia affects females and males equally.

Our results show that almost all β-thalassemia carriers have Portuguese nationality and present the same spectrum of common β-thalassemia mutations that was previously described in Portugal.[19] Only one different lesion, the Cd41/42 (-CTTT) deletion, was for the first time diagnosed here. On the contrary, the common 3.7kb α-thalassemia deletion was found in individuals with a larger range of nationalities. Heterozygous individuals for this condition are clinically asymptomatic, and present with slight hypochromia and eventually microcytosis or may have normal hematological findings. Even if inherited in the homozygous state, α^+-thalassemia is not considered significant in the screening programs and policies designed to detect couples at risk of hydrops fetalis.[22] However, although heterozygous and homozygous α^+-thalassemia individuals are clinically normal, and therefore do not require treatment, it is important to recognize the condition in order to elucidate the cause of microcytosis or hypochromia and not to confuse with and treat these cases as an iron deficiency condition.

We have observed that all the 54 individuals with α-thalassemia have hypochromia (100%) but only 29.6% have microcytosis. Therefore, we can conclude that the first parameter is more frequently associated with α-thalassemia than the latter. On the other hand, we have found that 21 out of the 22 carriers of β-thalassemia revealed microcytosis and hypochromia

simultaneously. It is known that these two parameters, along with an elevation of HbA2 (not measured in this study), are the typical hematological phenotype of the β-thalassemia carriers.[23] The only exception found in this study (1/22) was a man with β-thalassemia presenting with a very mild phenotype, without anemia or microcytosis (Hb = 14.5 g/dL, MCV = 82.3 fL), in whom just hypochromia was observed (HGM = 24.4 pg). However, this participant was a double heterozygote for β- and α-thalassemia. It is known that the co-inheritance of β- and α-thalassemia improves the hematological parameters, since it attenuates the disequilibrium between β- and α-globin chains, and consequently it may be a factor for misdiagnosis of β-thalassemia carriers. On the contrary, if a β-thalassemia carrier co-inherited a triple α-globin gene, the imbalance between β- and α-globin chains is increased and consequently the phenotype worsens and could even reach a thalassemia intermedia condition.[24]

Taking advantage of the small differences in the red cell indices between thalassemia trait and iron deficiency observed in CBC tests, several studies have developed mathematical formulas with the aim of discriminating those conditions.[25] Among them, the Red Cell Distribution Width Index (RDWI) = (MCV x RDW)/RBC, provides valuable help.[26,27] When its value is higher than its cut-off (> 220) it is suggestive of iron deficiency. In our study, the group of thalassemia carriers had a RDWI mean of 212.6 while the group without thalassemia had a RDWI mean of 261.2, suggesting the presence of iron deficiency in most of the participants of this latter group. To validate this hypothesis, further research should be carried out, including, for example, the measurement of serum ferritin and transferrin saturation.

Another limitation of this study consisted in the incapacity to detect the clinically relevant hemoglobin variants (namely HbS) in the general participants of INSEF. In fact, this study was not designed to detect hemoglobin structural variants since it is known that most of them are not associated with the hematological changes of microcytosis or hypochromia. Nevertheless, we have detected three cases of hemoglobin variants. Among them, one is a carrier of Hb S (*HBB*:c.20A>T) who has co-inherited an α-thalassemia allele ($-\alpha^{3.7kb}$). Consequently, this is the reason why this participant had a hypochromic anemia (Hb = 11.1 g/dL, MCH = 25.9 pg). Another participant was diagnosed as a carrier of Hb C (*HBB*:c.19G>A). This participant had no anemia or hypochromia but presented with mild microcytosis (MCV = 72.5 fL), which is in agreement with what is described for Hb C carriers in public databases.[28] The third hemoglobin variant was detected in a male, who revealed a microcytic anemia (Hb = 11.1 g/dL, MCV = 75.8 fL). He is a carrier of Hb D-Portugal (*HBB*:c.364G>C) but his hematological phenotype is worse than what is described in public databases for carriers of this variant.[29] This fact may be explained by a possible co-existence of iron deficiency anemia since his RDWI value was 256 (> 220). Therefore, in order to increase the knowledge about the prevalence rate of the clinically relevant structural variants in Portugal (especially of Hb S) further studies should be carried out, covering a larger sample of the population and including the biochemical characterization of the hemoglobin fractions present in fresh blood aliquots.

Our results also revealed that the thalassemia carriers are living in all the regions of Portugal, including both the mainland and the islands. Most of them self-reported their health as being fair or good, as expected for a clinically asymptomatic thalassemia carrier. However, and considering the β-thalassemia carriers (who have the typical hematological phenotype of microcytosis and hypochromia, and more than half also have anemia), only one of them self-reported anemia. We would like to draw attention to this fact, as it is suggestive that, probably, many of them are not aware of their genetic condition.

According to the guideline on hemoglobinopathies of the Directorate General of Health,[11] we would like to emphasize the role of primary health care services in identifying and managing the hereditary implications related to these genetic conditions. Furthermore, in addition to the availability of diagnostic genetic testing, we reinforce that for prevention and control of clinically severe disease cases, such as homozygosity or compound heterozygosity for β-thalassemia, prenatal molecular diagnosis with genetic counselling is available in Portugal.

CONCLUSION

The present study allowed us to conclude that α- and β-thalassemia traits are a considerable cause of microcytosis or hypochromia, with or without anemia, in individuals living in Portugal, since these genetic conditions were detected in 37% of the investigated cases. This information has clinical relevance because microcytosis and hypochromia are often interpreted as indicators of iron deficiency and, consequently, these individuals may be mistreated with oral iron therapy, and may end up not being diagnosed as thalassemia carriers. The latter may have serious consequences, particularly for β-thalassemia carriers, and their families, because they will be deprived of genetic counselling and may be at risk of having seriously affected offspring. We would like to draw attention and alert healthcare professionals, particularly family physicians, to this important matter.

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AUTHOR CONTRIBUTIONS

DS: Molecular diagnosis, conception of the work, statistical analysis and critical review the manuscript.

MB: Data acquisition, DNA extraction, conception of the work and critical review the manuscript.

IK: Database management, statistical analysis and critical review the manuscript.

JM, MPM: NGS experiments, bioinformatics analysis and critical review the manuscript.

PL: Molecular diagnosis and analysis of the results.

CMD: Conception of the work and critical review the manuscript.

PF: Conception of the work, analysis of the results, draft the manuscript and critical review the paper.

PROTECTION OF HUMANS AND ANIMALS

The authors declare that the procedures were followed according to the regulations established by the Clinical Research and Ethics Committee and to the Helsinki Declaration of the World Medical Association updated in 2013.

DATA CONFIDENTIALITY

The authors declare having followed the protocols in use at their working center regarding patients' data publication.

COMPETING INTERESTS

The authors have declared that no competing interests exist.

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ACTA MÉDICA PORTUGUESA
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A Collaborative Approach for the Development of a Standardized Set of Patient-Centered Outcomes in Head and Neck Cancers

Uma Abordagem Colaborativa para o Desenvolvimento de um Conjunto Padronizado de Resultados Centrados no Doente com Cancro de Cabeça e Pescoço

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ABSTRACT

Introduction: Head and neck cancers remain a significant health burden worldwide. Standardizing the care provided to these patients through the systematic measurement of established indicators is key to improve their outcomes. The aim of this study was to establish a relevant set of outcome indicators in this condition and identify measurement tools and requirements to do so.

Methods: One scientific committee and two regional working groups worked in a stepwise manner to narrow down an initial list of potential outcome indicators retrieved from an exhaustive literature review to a smaller set of outcome indicators according to their clinical practice. This was assessed by one representative of a head and neck cancer patient association until a final set of indicators was reached.

Results: A total of 164 outcome indicators comprising case-mix, outcomes, and adverse events dimensions were retrieved from the literature. These were reduced to a working set of 79 outcome indicators by the Scientific Committee and divided into seven categories including demographics, clinical status, tumor-related parameters, nutritional status, treatment, health and quality of life parameters and survival. Subsequently, these indicators were further reduced to a set of 50 indicators by the regional working groups and to a set of 49 indicators by the final Scientific Committee assessment. Finally, the discussed indicators were appraised by a head and neck cancer patient association, which added the 'rehabilitation' category, a key parameter to these patients.

Conclusion: An initial set of outcome indicators for head and neck cancer was systematically developed aiming to standardize the care provided to these patients across institutions at national level and identify measurement tools and requirements to measure those indicators. This standard set should be continuously improved and consistently adopted in the different clinical and national settings.

Keywords: Head and Neck Neoplasms; Patient-Centered Care; Patient Outcome Assessment; Quality Indicators, Health Care

RESUMO

Introdução: O cancro de cabeça e pescoço continua a ter um impacto considerável quer para o doente quer para os sistemas de saúde a nível mundial. Uniformizar os cuidados de saúde prestados a estes doentes, através da medição sistemática de indicadores estabelecidos, é fundamental para a melhoria contínua dos resultados em saúde. O objetivo deste estudo foi estabelecer um conjunto relevante de indicadores de resultados para o cancro de cabeça e pescoço e identificar ferramentas de medição e respetivos requisitos para a sua realização.

Métodos: Através de uma revisão exaustiva na literatura, obteve-se uma lista inicial de potenciais indicadores de resultados para o cancro de cabeça e pescoço. Um comité científico e dois grupos de trabalho regionais trabalharam em colaboração para reduzi-la por forma a obter um conjunto de indicadores ajustado à sua prática clínica. Esta lista foi depois avaliada por um representante de uma associação de doentes de cancro de cabeça e pescoço alcançando-se um conjunto final de indicadores.

Resultados: Da revisão literária, um total de 164 indicadores foram identificados abrangendo as dimensões de *case-mix*, resultados e eventos adversos. Estes foram, posteriormente, reduzidos a um conjunto de 79 indicadores pelo comité científico e divididos em sete categorias, incluindo demografia, estado clínico, parâmetros relacionados com o tumor, estado nutricional, tratamento, parâmetros de saúde e qualidade de vida, e sobrevida. Subsequentemente, essa lista foi ainda encurtada para 50 indicadores, pelos grupos de trabalho regionais, e reduzida para 49 indicadores pela avaliação final do comité científico. Por fim, os indicadores discutidos foram avaliados por um representante da associação de doentes, que acrescentou a categoria, 'reabilitação', parâmetro fundamental para estes doentes.

Conclusão: Um conjunto inicial de indicadores de resultados para cancro de cabeça e pescoço foi definido com o objetivo de padronizar a prática clínica a nível nacional e identificar as ferramentas de medição e os requisitos necessários para os medir. Este conjunto de indicadores deve ser continuamente melhorado e adotado de forma consistente nos diferentes contextos clínicos a nível nacional.

Palavras-chave: Avaliação de Resultados na Ótica do Doente; Cuidados Centrados no Doente; Cuidados de Saúde; Indicadores de Qualidade; Neoplasias da Cabeça e Pescoço

INTRODUCTION

According to the latest GLOBOCAN estimates, head and neck (H&N) cancer is the 8th most common cancer worldwide in terms of both incidence and mortality. With 450 000 deaths every year, the disease represents a significant global health burden.[1] Head and neck squamous cell carcinoma (HNSCC) is the most common histological

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subtype[2] and comprises a biologically and clinically heterogeneous group of tumors from the oral cavity, pharynx (oropharynx, hypopharynx and nasopharynx), larynx, sinonasal cavity and salivary glands, with disparate risk factors, molecular pathogenesis, treatment response, and prognosis.[3,4]

Well-established risk factors include tobacco and alcohol consumption, but oncogenic viruses as the human papillomavirus (HPV) are emerging as new etiological agents associated with oropharyngeal squamous cell carcinoma.[5] Additionally, also the microbiome and diet have been implicated as contributing factors in recent decades.[6]

The treatment strategy in H&N cancers aims to achieve the highest cure rate with the lowest morbidity risk. To achieve this, patients should undergo pre-treatment risk assessment incorporating both objective tumor parameters (such as tumor location, histology and TNM stage) and patient parameters (physiological age, comorbidities, nutritional status, previous history of cancer, occupation, expected functional outcome, personal preference). Disease management requires a multidisciplinary approach involving several medical specialties, as well as multimodal treatment involving surgery, radiation therapy, chemotherapy and, more recently, immunotherapy, each with trade-offs between treatment outcomes and quality of life (QoL).[7–11] Additionally, the multidisciplinary nature of the management approach of these patients can make it challenging to define the most relevant patient outcomes to consider and define a unified and robust treatment strategy.

In Europe, five-year survival rates of patients with head and neck tumors are poor and vary according to subsite: 61% for laryngeal, 49% for oral cavity, 41% for oropharyngeal, and 25% hypopharyngeal squamous cell carcinoma (SCC).[12] These suboptimal survival rates highlight the need for improved risk stratification to identify patients at higher risk of recurrence and tailor treatment for them.

The high prevalence of loco-regional recurrence and/or metastatic disease is responsible for the high mortality rates reported in HNSCC.[13] Indeed, patients with early disease stages carry a favorable prognosis, with five-year survival rates close to 80%, while for patients with locally advanced disease this rate is below 50%.[14]

Significant regional and between-hospital heterogeneity exists in the treatment patterns and quality of care delivered to these patients, with direct impact on their health outcomes. In addition to heterogeneous treatment practices, hospitals also use different quality indicators. Quality indicators are used by healthcare institutions to evaluate the quality of care provided and identify areas for improvement, but many indicators focus on treatment process and structure rather than on their relevance to patient outcomes. Besides not always having an impact on relevant patient outcomes, the registry of these indicators represents a significant burden for clinicians. Additionally, the lack of standardization in indicators collected across hospitals and of routine collection of such indicators limits the ability to retrieve useful insights into the quality of care provided nationally. Systematic outcome measurement is the cornerstone of value improvement and key in guiding improvement efforts and value-based reimbursement models in health care[15–17] and should be used as the basis to ensure high-value health care for all patients.

The conceptual framework of value-based health care is increasingly being used to improve the quality of care delivered to patients. This strategy is based on the premise that the value of health services, rather than the volume of services, is the most relevant indicator of the quality of care provided, with 'value' defined as the patient-relevant outcomes achieved relative to its costs over the full cycle of care.[18,19] The foundation of this strategy is a common definition of value, starting with outcomes, which should be patient-centered and include not only survival, but also the impact of the disease and its treatment on patients' quality of life and ability to live productive lives free of treatment or disease symptoms. In this sense, outcome indicators should integrate both established disease control measures and patient-reported outcome measures (PROMs).

Implementing a strategy of value-based health care for a specific medical condition requires the definition of a relevant set of outcome indicators relevant to both patients and stakeholders and respective collection and measurement using well-defined and standardized metrics. However, defining and measuring health outcomes is challenging, as they should encompass not only survival and overall disease control, but also treatment complications and health-related quality of life (HRQoL) during and after treatment. Efforts to measure and report health outcomes have been developed for some malignancies, such as lung, breast, colorectal, and prostate cancers, but no recommended set of outcomes exists for head and neck cancers.[20–25]

The primary aim of this initiative was to establish a Portuguese consensus on a relevant set of outcome indicators for head and neck cancers that could enable the standardization of the care provided to these patients across institutions at a national level and identify measurement tools and requirements to measure those indicators. Ultimately, the project aims to build evidence on head and neck cancers and thus improve the quality of care delivered to these patients.

METHODS

Working group

A multidisciplinary working group of 26 experts convened to develop a standardized set of outcome indicators for head and neck cancers. This group was organized in

three multidisciplinary teams: one scientific committee (corresponding to the study authors) and two regional workgroups (see the Acknowledgements section).

The Scientific Committee was composed of seven representatives of Medical Oncology, Surgical Oncology, Radiation Oncology, Otolaryngology, Nutrition, and Hospital Pharmacy and was responsible for project leadership and for providing guidance along its several phases.

Regional Workgroups consisted of two groups of eleven experts from the North and eight experts from the South of Portugal, responsible for advising and providing input on the relative importance of outcomes and respective ease of implementation in clinical practice. Regional workgroups comprised representatives from the same clinical areas represented in the Scientific Committee plus Nursing, Maxillofacial Surgery, and Rehabilitation Medicine.

Outcome indicator selection procedure

The definition of a standard set of indicators was a multidisciplinary process implemented through a stepwise approach composed of five phases (Fig. 1).

Phase 1 consisted of a literature review and initial selection of a comprehensive set of outcome indicators. In Phase 2, the Scientific Committee evaluated and discussed the comprehensive set of indicators retrieved from the literature and narrowed it down to a working set of indicators. In Phase 3, the indicators selected by the Scientific Committee were analyzed, discussed, and adjusted by the Regional Workgroups. Phase 4 consisted of a final expert round, in which the Scientific Committee evaluated and discussed the proposals of the Regional Workgroups. Finally, in Phase 5 a head and neck cancer patient association reviewed, commented, and suggested new outcome indicators to the Committees' list, which led to a final standard set of outcome indicators in head and neck cancers.

Phase 1 | Literature review and initial selection of a comprehensive set of potential outcome indicators

A structured literature review was conducted to retrieve an initial comprehensive set of potential outcome indicators. The literature search was based on (i) outcome standard sets previously defined for other malignancies by various entities, including the International Consortium for Health Outcomes Measurement (ICHOM), (ii) head and neck cancer-related clinical trials and studies, (iii) and identification of clinical outcomes, patient-reported outcomes (PROs), and measures of HRQoL in patients with head and neck cancer and respective measurement tools and frequency. The search included all types and stages of head and neck cancer.

Based on data retrieved from this literature review, indicators and measurement tools identified were categorized and compared with those referred in studies to corroborate their relevance for inclusion.

The retrieved results were systematized, and a preliminary comprehensive set of potential outcome indicators was obtained and conveyed to the Scientific Committee.

Phase 2 | Initial Scientific Committee meeting – Evaluation and initial filtering of the comprehensive set of outcome indicators

Phase 2 comprised two steps. In the first step, the members of the Scientific Committee independently analyzed the comprehensive set of potential outcome indicators, rating them on a 10-point Likert-type scale according to their importance in clinical practice and disease management (1 being the least important and 10 the most important) while simultaneously assessing their relevance for the different head and neck cancer sites. In this step, Scientific Committee experts had the opportunity to add new outcome indicators to the predefined list and suggest outcome clustering, changes, or exclusions. The level of consensus of the

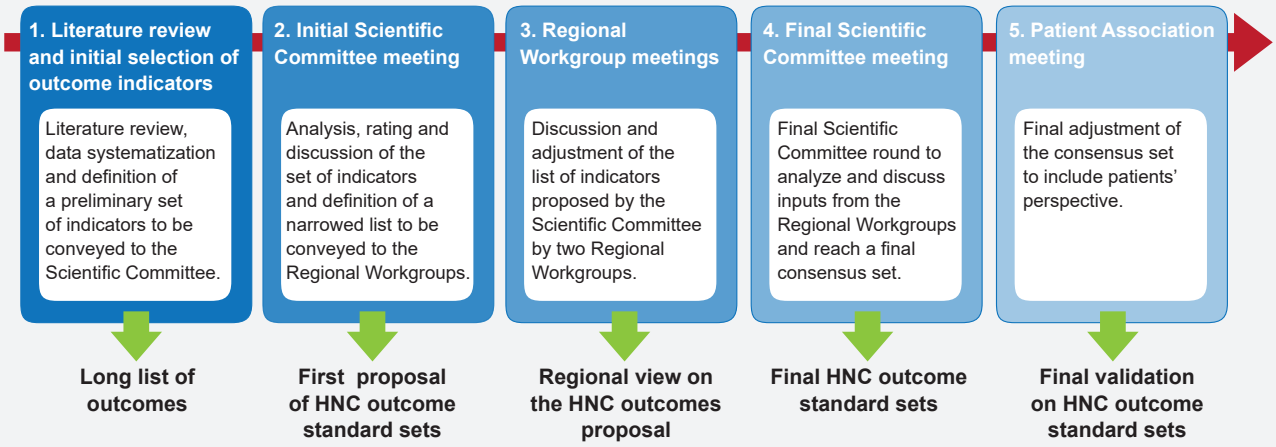

Figure 1 – Stepwise approach to the selection of a standard set of outcome indicators in head and neck cancers

experts was then evaluated, and indicators were clustered according to their relative importance levels: (i) 'high importance' – indicators rating between 9 and 10, (ii) 'medium importance' – indicators rating between 7 and 8, and (iii) 'low importance' – indicators rating between 1 and 6.

In the second step, the Scientific Committee convened to analyze and discuss the results from the previous step. Indicators clustered as 'high importance' were swiftly validated and included in the standard set. The remaining were individually discussed and assessed for the need to be grouped, changed, or excluded. The Scientific Committee also had the opportunity to add new indicators if relevant for the disease treatment or management. A Delphi methodology was applied in this step.

At the end of Phase 2, a set of outcome indicators was obtained and conveyed to the Regional Workgroups for evaluation and discussion.

Phase 3 | Regional Working Group meetings – Evaluation and discussion of the set of outcome indicators

In Phase 3, the set of outcome indicators proposed by the Scientific Committee was discussed by two Regional Working Groups. Similarly to Phase 2, experts from each working group previously analyzed individually the list of indicators according to their relative importance on a 10-point Likert-type scale (from 1 – least important to 10 – most important) and according to their feasibility of implementation in clinical practice on a 5-point Likert-type scale (from 1 – very difficult to implement to 5 – very easy to implement). They then convened and a Delphi methodology was also applied, whereby outcome indicators were evaluated individually. Similarly to Phase 2, the level of expert consensus was also assessed. Indicators that scored between 9 and 10 in the importance rating and 5 in the feasibility of implementation rating were swiftly validated and included in the standard set. The remaining indicators were individually discussed and assessed for the need to be grouped, changed, or excluded.

At the end of Phase 3, a third set of outcome indicators was retrieved and conveyed to the Scientific Committee for final validation.

Phase 4 | Final Scientific Committee meeting – Definition of the final standard set of outcome indicators

Phase 4 consisted of a final expert round with the Scientific Committee to analyze and discuss inputs from the Regional Working Groups and reach a final consensus on a standard set of outcome indicators to be implemented in the management of patients with head and neck cancers in the future.

Additionally, several aspects related to the selected indicators were also discussed at this phase, including their category, preferred designation, definition, measure/response options, metrics, inclusion criteria (i.e., if the indicator is applicable to all patients or to specific head and neck cancer subtypes), time of collection, and reporting source.

Phase 5 | Patient Association meeting – Final validation of the standard set of outcome indicators

The fifth and final phase of the process sought to include the perspective of patients, besides that of the medical community, in an integrated approach to the definition of outcome measures in head and neck cancers. To do this, an interview was conducted with a member of a head and neck cancer Patient Association to retrieve his feedback on the predefined list of indicators, in which he had the opportunity to comment and/or add indicators valued by head and neck patients.

RESULTS

The progress along the five phases of this project regarding selection of indicators is depicted in Fig. 2.

Phase 1

The literature search identified six randomized clinical trials and 60 studies in head and neck cancer, as well as four reports on standard outcome sets previously defined for other malignancies. All literature sources were reviewed until a saturation of outcome indicators was achieved at 164 indicators, which comprised the initial comprehensive set of outcome indicators conveyed to the Scientific Committee. The retrieved indicators were divided in three dimensions with several categories each: (i) case-mix (ii) outcomes and (iii) adverse events (Table 1).

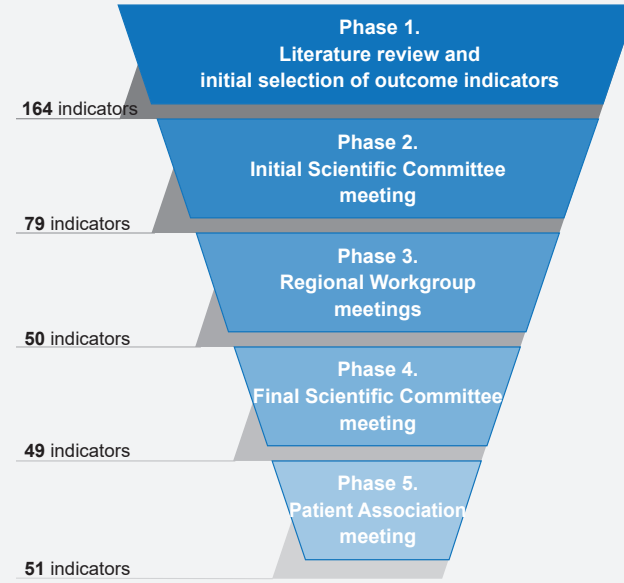

Figure 2 – Selection of head and neck cancers outcome indicators throughout the different phases of the project

Table 1 – Initial comprehensive set of outcome indicators retrieved from the literature search (section 1 of 4)

Dimension	Category	Indicator
Case-mix	Demographics	Age
		Gender
		Ethnicity
		Place of residence
		Smoking history
		Alcohol history
		Education level
		Employment situation
		Income
		Private health insurance
		Marital status
		Family history of cancer
	Tumor-related	Tumor site
		TNM status
		Stage of disease
		Recurrence/Metastases
		Histological type
	Baseline clinical status	Human papilloma virus
		p16 status
		Malignant lesions
		Performance status
		Tumor markers
		Anemia
		Mental illness at diagnosis
		Dysphagia
		Comorbidities
	Nutritional status	Weight loss
		Body mass index
		Nutritional status
		Muscle mass
		Diet type
Outcomes	Survival	Overall survival
		Progression-free survival
		Death related to cancer
		Death unrelated to cancer
	Treatment	Type of treatment
		Treatment intent
		Radiation dose
		Tumor response
		Adverse effects
		Treatment compliance

Table 1 – Initial comprehensive set of outcome indicators retrieved from the literature search (section 2 of 4)

Dimension	Category	Indicator
	Clinical status	Performance status
		Spinal accessory nerve function
		Perioperative complications
		Shoulder capacity
	Health and quality of life	Overall well-being
		Physical functioning
		Social functioning
		Emotional functioning
		Cognitive functioning
		Fatigue and vitality
		Pain
		Activity
		Shoulder capacity
		Mood
		Anxiety
		Recreation
		Shortness of breath
		Nausea and vomiting
		Loss of appetite
		Insomnia
		Constipation
		Diarrhea
		Economic difficulties
		Personal care
		Weight changes
		Swallowing problems
		Problems with taste/smell
		Oral cavity problems
		Trouble speaking
		Problems with social meals
		Sexual problems
		Cough
		Aphonia
		Appearance
		Eating problems
		Use of analgesics
		Use of oral nutritional supplements
		Need for tube feeding
		Smoking habits
		Alcohol consumption
		Emotional functioning

Table 1 – Initial comprehensive set of outcome indicators retrieved from the literature search (section 3 of 4)

Dimension	Category	Indicator
	Nutritional status	Nutritional status
		Weight
		Fat percentage
		Body composition
		Tube feeding complications
Adverse events	Hematology	Neutropenia
		Anemia
		Thrombocytopenia
		Leukopenia
		Myelosuppression
		Febrile neutropenia
		Thrombosis
	Parameter variation	Weight loss
		ALT increase
	Chemistry	Hypomagnesemia
		Hypokalemia
		Hyponatremia
	Cardiovascular disorders	Hemorrhage
		Epistaxis
		Atrial fibrillation
	Skin disorders	Skin reaction/dermatitis
		Pruritus
		Dry skin
		Skin reaction to injection
		Hyperpigmentation
	Metabolism and nutrition disorders	Dehydration
		Anorexia
		Loss of appetite
	Reproductive system disorders	Erectile dysfunction
	Gastrointestinal disorders	Sickness
		Mucositis
		Dysphagia
		Diarrhea
		Vomiting
		Constipation
		Sensitive tongue, saliva accumulation
		Defective salivary incontinence
		Dyspepsia

Phase 2

At this phase, the comprehensive list of outcome indicators retrieved from the literature review was initially reduced to 159 potential outcome indicators stemming from the individual analysis of Scientific Committee members. A total of 77 outcome indicators with high importance (rate of importance 9 − 10), 71 indicators with medium importance (rate of importance 7 − 8), and 11 indicators with low

Table 1 – Initial comprehensive set of outcome indicators retrieved from the literature search (section 4 of 4)

Dimension	Category	Indicator
	General disorders	Fatigue
		Fever/Pyrexia
		Pain
		Flu-like symptoms
		Mouth pain
		Apathy
		Trismus
		Hyposalivation (xerostomia)
	General disorders and administration site reactions	Infusion reactions
	Immunological disorders	Infection
		Paronychia
	Musculoskeletal disorders	Fibrosis
		Arthralgia
		Osteonecrosis
	Neurological disorders	Dizziness
		Dysgeusia
		Partial seizures
		Insomnia
	Eye disorders	Conjunctivitis
	Dental disorders	Stomatitis
		Radiation caries
	Renal and urinary disorders	Kidney failure
	Respiratory disorders	Dyspnea
		Cough
	Other/Not classified	Muscle mass loss
		Nephrotoxicity
		Neurotoxicity
		Laryngeal toxicity
		Ototoxicity
		Skin toxicity
		Hematologic toxicity
		Ulcers
		Suppuration
		Odor
		Swelling
		Gastrointestinal perforation
		Wound complications
		Cheilitis
		Muscle spasms
		Palmoplantar erythrocytosis
		Pneumonia
		Death caused by toxicity
		Hyperthyroidism
		Hypothyroidism

importance (rate of importance 1 − 6) were rated. These were subsequently discussed and readjusted according to the experts' opinion and clinical practice. The main changes to the initial set retrieved from Phase 1 were the grouping of 'clinical basal' and 'clinical status' categories in a single one named 'clinical status' within the 'baseline' dimension, and the exclusion of the 'adverse events' dimension and its incorporation in the 'treatment' category within the 'outcomes' dimension. This resulted in a working set of 79 outcome indicators at the end of Phase 2, organized in two main dimensions: (i) baseline, including demographics, clinical status, tumor-related parameters, and nutritional status, and (ii) outcomes, including survival, treatment, and health and quality of life parameters [Table 1 in Appendix 1 (Appendix 1: https://www.actamedicaportuguesa.com/revista/index.php/amp/article/view/18180/15008)]. This working set of 79 indicators was conveyed to the Regional Workgroups.

Phase 3

From the working set of 79 outcome indicators selected by the Scientific Committee, 10 were excluded, 32 were grouped, and 13 were added by Regional Workgroups, resulting in a set of 50 indicators at the end of Phase 3 [Table 2 in Appendix 1 (Appendix 1: https://www.actamedicaportuguesa.com/revista/index.php/amp/article/view/18180/15008)]. To reach this set of indicators, the main adjustments performed by the Regional Workgroups to the list of outcome indicators conveyed by the Scientific Committee were (i) grouping of socioeconomic indicators into a new indicator designated 'socioeconomic status'; (ii) grouping of the several nutritional status indicators in a single indicator designated 'nutritional status'; (iii) restructuring of 'health and quality of life' indicators in two new indicators designated 'overall functional status' and 'overall quality of life'; (iv) grouping of oral evaluation indicators in a single indicator designated 'oral pre-malignant lesions'; (v) grouping of disease stage and TNM status in a single indicator designated 'disease stage'; (vi) grouping of 'type of treatment' and 'therapeutic approach' indicators in a single one designated 'therapeutic approach'; and (vii) inclusion of 'airway complications', 'stomatological assessment', 'dysphonia', 'recurrence-free survival', 'treatment adherence', 'surgical approach', and 'ostomy complications' as new indicators.

Phase 4

The set of 50 outcome indicators selected by the Regional Working Groups were again discussed and adjusted by the Scientific Committee, resulting in a final set of 49 indicators. This set was conveyed to the Patient Association for final assessment and validation [Table 3 in Appendix 2 (Appendix 2: https://www.actamedicaportuguesa.com/revista/index.php/amp/article/view/18180/15009)].

Phase 5

In the last phase of the project, the perspective of patients with head and neck cancer was incorporated in the previously defined set, resulting in a final set of 51 outcome indicators [Table 3 in Appendix 2 (Appendix 2: https://www.actamedicaportuguesa.com/revista/index.php/amp/article/view/18180/15009)]. The main adjustment made was inclusion of the category 'rehabilitation', as many patients will require some type of rehabilitation along their patient journey (e.g., speech, feeding, social, psychological rehabilitation, among others). The 'rehabilitation' category included comprised two indicators: 'need for rehabilitation' and 'rehabilitation initiation date', which intends to account for the time between diagnosis and rehabilitation start, a key parameter in the rehabilitation progress of patients.

The need to include the caregiver's perspective on the 'overall quality of life' indicator was also mentioned, since patients can experience difficulties in objectively perceiving their status and the voice of caregivers is important for an external and more integrative view of the daily reality of patients.

Overall, patients considered this initiative extremely relevant to eradicate the disparities in the management of head and neck cancers.

DISCUSSION

The rise in healthcare costs and treatment advances have emphasized the importance of value-based health care. This framework is increasingly being acknowledged in the provision of health services, as it has the potential to substantially improve patient outcomes.[26]

The standardization of outcome measures that are meaningful to patients is intrinsic to this value-based approach but has been a major challenge that only recently started to be addressed in several medical conditions. In head and neck cancers, it represented an unmet need, emphasized by the fact that this is a complex disease that requires a multidisciplinary approach from several medical specialties that do not always communicate in an optimal way towards the best patient management. The present collaborative approach set out to tackle this unmet need.

The development of a standardized set of patient-centered outcomes in head and neck cancers was a national project that convened a multidisciplinary working group. The standard set of outcome indicators retrieved at the end of the project was based on a literature review and clinical and patient input and is believed to capture key outcomes relevant to patients with head and neck cancer over the full cycle of care, from diagnosis to treatment completion, survivorship, and rehabilitation. Its implementation will enable health institutions and practices to restructure health care delivery based on a value-centered approach.

The project sought to aggregate several medical specialties participating in head and neck cancer management and also include the perspective of patients on the outcomes that matter to them, in an effort to obtain an integrated vision of a standard set of outcomes to be systematically measured in all patients with this condition. The retrieved consensus comprised 51 indicators from eight different outcome hierarchy levels: demographics, clinical status, tumor-related characteristics, nutritional status, treatment, survival, rehabilitation, and health and quality of life, which should be routinely implemented in clinical practice.

The set of indicators devised from this project represents a proof-of-concept and intends to pave the way for broader adoption and for endorsement by national policies and regulatory entities. Although randomized controlled trials remain the gold standard for comparison of treatment outcomes, outcome measurement in routine clinical practice can better reflect outcomes in a real-life setting and have a more direct impact for patients.

Centers are now encouraged to implement the standard set-in healthcare institutions and systematically collect that information in the clinical practice, so that health care delivery to these patients can be improved in years to come. This can be done in a stepwise manner, beginning with retrieval of a small group of indicators and subsequently expanding it to include a larger number. This structured data collection has several challenges, as it will require investment in human resources and information technology, training clinical staff, and redesigning the clinical workflow. Most importantly, it will require a change in clinical attitudes. However, its implementation, not only at the point of care but also for retrospective and comparative analyses, is key for quality improvement within health institutions and to generate evidence.

The main strength of this study is the combination of the perspective of a relevant team of experts and a patient association related to head and neck cancers to obtain the most accurate set of indicators relevant for patients. This cross-disciplinary effort improves the consistency of data and their relevance to patients, besides health services. The main limitation is that this project is a proof-of-concept, and its output represents a starting point in the definition of a true global outcome standard set in head and neck cancers. Therefore, it should be subject to additional and regular discussion, review, and adjustments.

The experience of collecting these outcomes in clinical practice will be important to understand their applicability and make any necessary adjustments. In the future, they should be updated, ideally on an annual basis, based on feedback from implementing Centers and developments in the field of head and neck cancer.

CONCLUSION

The aim of this initiative was to develop a standardized set of patient-centered outcome indicators to be systematically used during disease management to evaluate the quality of care in head and neck cancers across disease management and patient journey. Through a literature review and clinical and patient inputs, a set of indicators was achieved, which represents a proof-of-concept to be further validated and widespread adopted in the different clinical settings. The routine and systematic collection of these indicators will allow monitoring and comparing head and neck cancer patient outcomes within and across institutions and improve them in the long term.

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AUTHOR CONTRIBUTIONS

All authors report substantial contributions to the conception of the work; Drafting the work and revising it critically for important intellectual content; Final approval of the version to be published; Agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are

appropriately investigated and resolved.

PROTECTION OF HUMANS AND ANIMALS

The authors declare that the procedures were followed according to the regulations established by the Clinical Research and Ethics Committee and to the Helsinki Declaration of the World Medical Association updated in 2013.

DATA CONFIDENTIALITY

The authors declare having followed the protocols in use at their working center regarding patients' data publication.

PATIENT CONSENT

Obtained.

COMPETING INTERESTS

AJ, ARN, CA, GV, LR, PA: Received from Bristol Myers Squibb payment for the participation of standard set definition work sessions. Received from IQVIA support under the form of medical writing.

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ACTA MÉDICA PORTUGUESA
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Adjuvant Chemotherapy De-Escalation with Genomic Assay Protocol in Patients with Early Breast Cancer: A Single-Centre Prospective Cohort Study

Redução de Quimioterapia Adjuvante com Utilização de Teste Genómico em Doentes com Carcinoma da Mama Localizado: Estudo de Coorte Prospetivo Unicêntrico

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ABSTRACT

Introduction: Genomic assays are useful tools for tailoring adjuvant treatment in early breast cancer. We aimed to analyse the role of an institutional protocol of a genomic assay for chemotherapy de-escalation.

Methods: Prospective cohort study of all consecutive women diagnosed with hormone receptor-positive and human epidermal growth factor receptor 2-negative early breast cancer, tested with the 21-gene Recurrence Score (RS) assay from August 2015 to July 2018 at a Portuguese cancer centre. For being tested, patients should meet at least one of the pre-defined inclusion criteria: i) luminal A-like, pT2pN0; ii) luminal A-like, 1 – 3 positive nodes and comorbidities with higher risk of chemotherapy-induced toxicity; iii) pT1-2pN0, progesterone receptor ≤ 20% and/or Ki67 14% – 40%. Adjuvant treatment was de-escalated to isolated endocrine therapy if RS was less than 18. We measured the reduction in chemotherapy prescribing and its clinical impact, the RS association with pathologic features, and the protocol feasibility.

Results: We tested 154 women with a median age of 61 years old (range: 25 – 79), 69% postmenopausal. Tumours were mainly pT1 (55%), pN0 (82%), invasive ductal (73%), G2 (86%), luminal B-like (69%) and stage IA (85%). We obtained a RS less than 18 in 60% of women, with an overall adjuvant chemotherapy reduction of 65%. Seven (95% confidence interval: 5 – 10) patients needed to be screened with the 21-gene RS assay to prevent one clinically relevant adverse event during the first six months of adjuvant treatment. Considering the currently used RS cut-off, only 9% of node-negative and 11% of node-positive patients had RS over 25. We found no relevant associations between RS and pathologic features. The protocol was feasible and did not compromise the adequate timing for adjuvant treatment.

Conclusion: These criteria allowed the de-escalation of adjuvant systemic treatment in at least six out of ten women.
Keywords: Antineoplastic Agents, Hormonal; Breast Neoplasms; Chemotherapy, Adjuvant; Gene Expression Profiling; Precision Medicine

RESUMO

Introdução: As análises genómicas têm personalizado o tratamento adjuvante em cancro de mama localizado. O objetivo deste estudo foi avaliar o impacto de um protocolo institucional de análise genómica para de-escalação de quimioterapia.

Métodos: Estudo de coorte prospetivo de todos os casos consecutivos de carcinoma da mama localizado com expressão positiva de receptores hormonais e sem sobre-expressão de *human epidermal growth factor receptor* 2, submetidos a um teste de quantificação de expressão de 21 genes para avaliação de *score* de recorrência (RS) entre agosto de 2015 e julho de 2018 num centro oncológico português. Para serem testadas, as doentes teriam de cumprir pelo menos um dos seguintes critérios de inclusão: i) *luminal* A-*like*, pT2pN0; ii) *luminal* A-*like*, 1 – 3 gânglios positivos e comorbilidades que constituam um maior risco para toxicidade induzida por quimioterapia; iii) pT1-2pN0, PR ≤ 20% ou Ki67 14% – 40%. O tratamento adjuvante foi de-escalado para hormonoterapia isolada quando o RS foi inferior a 18. Foi medida a taxa de redução de prescrição de quimioterapia e o seu impacto clínico, a associação do RS com características patológicas e a exequilidade do protocolo.

Resultados: Testámos 154 mulheres com mediana de idade de 61 anos (mínimo – máximo: 25 – 79), 69% pós-menopáusicas. Os tumores eram maioritariamente pT1 (55%), pN0 (82%), subtipo ductal invasivo (73%), G2 (86%), *luminal* B-*like* (69%) e estadio IA (85%). Obtivemos RS inferior a 18 em 60% das mulheres, com uma taxa de redução global de quimioterapia adjuvante de 65%. Esta análise genómica preveniu um evento adverso clinicamente relevante durante os primeiros seis meses de tratamento adjuvante por cada sete (intervalo de confiança 95%: 5 – 10) mulheres testadas. Considerando o *cut-off* mais recente para o RS, apenas 9% tiveram RS superior a 25, sendo que 11% das doentes com doença ganglionar teve RS superior a 25. Não houve correlação relevante entre RS e características anatomopatológicas. O protocolo não comprometeu o início atempado do tratamento adjuvante.

Conclusão: Este protocolo evitou a exposição a quimioterapia em pelo menos seis em cada dez mulheres.
Palavras-chave: Antineoplásicos Hormonais; Medicina de Precisão; Neoplasias da Mama; Perfilação da Expressão Génica; Quimioterapia Adjuvante

INTRODUCTION

Breast cancer is the most frequent malignancy and the leading cause of cancer related mortality in women.[1] About two-thirds of breast cancer cases are hormone receptor-positive and human epidermal growth factor receptor 2-negative (HR+/HER2-).[2] Immunohistochemistry allows to define a surrogate of two molecular subclasses of HR+/HER2- tumours, luminal A-like and luminal B-like, the latter when progesterone receptor (PR) expression < 20% and/or Ki67 ≥ 20%.[3] However, the clinicopathologic features do not accurately distinguish the patients who benefit from

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adjuvant chemotherapy.

The OncotypeDx™ Recurrence Score (RS) is the result of a genomic assay of a panel of 21 genes developed to analyse the tumour and to assess gene activity through RNA expression profiling. RS was initially divided into three groups based on retrospective analyses of prospective trials: i) low-risk of recurrence, RS < 18; ii) intermediate-risk, RS 18 – 30; iii) high-risk, RS ≥ 31.[4] OncotypeDx was later prospectively validated in two trials that recruited women with HR+/HER- early breast cancer: i) TAILORx for node-negative,[5] ii) RxPONDER for node-positive.[6] The TAILORx trial divided patients with node-negative HR+/HER2- disease into three categories of recurrence risk: low-risk (RS 0 – 10); medium-risk (RS 11 – 25) and high-risk (RS 26 – 100). Patients with RS 0 – 10 were spared from adjuvant chemotherapy and had similar outcomes of recurrence at five years.[7] The TAILORx analysis at nine years of follow-up showed that endocrine therapy was not inferior to chemoendocrine therapy in the group of women with RS 11 – 25. However, younger women (aged under 50 years old) with RS > 15 benefited from chemotherapy with a significantly lower risk of recurrence. These findings can be the consequence of estrogen deprivation from the chemotherapy-induced failure of ovarian function, since only 13% of the patients aged under 50 years old and treated with isolated endocrine therapy were given concomitant ovarian suppression.[5]

The RxPONDER trial[6] randomised patients with RS ≤ 25 to receive endocrine therapy alone or chemoendocrine therapy, and showed no benefit with the addition of chemotherapy in postmenopausal women with 1 – 3 positive nodes. In premenopausal women, chemotherapy was protective, with a five-year invasive disease-free survival of 89.0% in the endocrine therapy group and 93.9% in patients receiving chemoendocrine therapy (HR 0.60; 95% CI, 0.43 to 0.83), with a similar benefit in distant relapse-free survival (HR 0.58; 95% CI 0.39 to 0.87). Contrary to TAILORx, the RxPONDER trial failed to demonstrate the predictive value of RS for chemotherapy benefit in node-positive breast cancer. However, both studies agreed with the lack of chemotherapy benefit in postmenopausal women with zero to three positive nodes HR+/HER2- breast cancer and RS ≤ 25. Regarding premenopausal patients, women with node-negative disease can be spared from chemotherapy if RS < 15, but node involvement in premenopausal patients is associated with a chemotherapy benefit that is independent of RS.

Associations between the clinicopathologic features and RS have been explored, namely histology and Ki67. Some authors consider that well-differentiated tumours with favourable histologic subtypes might not need to be tested, since these features are associated with lower RS.[8,9] On the other hand, Ki67 expression has been reported as a strong individual prognostic factor,[10] with correlation with RS.[11] However, contradictory data supports no correlation of RS with Ki67 and conventional prognostic markers.[12]

The aim of this study was to evaluate the proportion of early breast cancer cases with adjuvant treatment de-escalation using a 21-gene RS assay protocol at a Portuguese cancer centre. As secondary objectives, we explored the clinical impact and the feasibility of this protocol. As exploratory objectives, we interpreted the results according to the TAILORx and the RxPONDER trials and measured the relationship between RS and histology or Ki67.

METHODS

Study design and setting

Prospective cohort study of all consecutive women diagnosed with stage I-II, HR+/HER2- invasive breast cancer, who performed tumour analysis with the 21-gene RS assay at Instituto Português de Oncologia de Lisboa Francisco Gentil, from August 2015 to July 2018. This is one of the largest cancer centres in Portugal, serving a geographical area of about four million inhabitants, and receiving nearly 14 000 new cancer cases per year. Of these, around 800 are newly diagnosed breast cancer cases, and about 60% receive chemotherapy. The manuscript was prepared according to The Strengthening the Reporting of Observational Studies in Epidemiology statement.[13]

Data source and ethical considerations

We used anonymous data prospectively collected from the electronic health records since protocol implementation. This institutional guideline protocol was reviewed and approved by the Ethics Committee of Instituto Português de Oncologia de Lisboa Francisco Gentil. Informed consent was not required due to the nature of the study (observational study in an academic hospital). We excluded all patients whenever refusal to participate with clinical data for investigational purposes was written in medical records.

Cohort

From August 2015, it was prospectively defined according to the institutional protocol that the 21-gene RS assay would be available for women with HR+/HER2- early breast cancer and at least one of the following criteria: i) Luminal A-like, pT2pN0; ii) Luminal A-like, 1 – 3 involved axillary nodes and comorbidities or performance status (PS) that put patients at high risk of chemotherapy-induced toxicity; iii) pT1-2pN0, PR ≤ 20% and/or Ki67 14% – 25%. Women included with more than one inclusion criterion were analysed separately. The upper Ki67 cut-off for inclusion was revised to 40% in April 2017, after an interim analysis (Martins-Branco, oral communication). All patients were

discussed at the multidisciplinary team meeting and the test was requested either at that time or later during the first appointment with the medical oncologist. The estimated time from sample shipment to the result was seven to 10 days (central laboratory). The period of patient inclusion for this analysis was closed in July 2018, when the final results of TAILORx trial were published,[5] which led to modifications of the RS cut-offs for adjuvant treatment recommendations. All tumour samples were locally reviewed by a single pathologist.

Outcomes

The study's primary outcome was the impact of the institutional protocol on the adjuvant treatment decision – proportion of patients with adjuvant treatment de-escalation: overall, per protocol, and by inclusion criterion. At the time of institutional protocol implementation, the treatment recommendations were: i) RS < 18 – isolated adjuvant ET; ii) RS ≥ 18 – adjuvant chemoendocrine therapy. All these patients would have been previously proposed for adjuvant chemoendocrine therapy according to the prior institutional treatment protocol.

As secondary clinical outcomes, we reported the cumulative incidence of recurrence and mortality until March 15th, 2022, clinically relevant adverse events (CRAE) occurring during the first six months of adjuvant treatment (unscheduled medical visits, hospital admissions, grade 3 febrile neutropenia as defined by Common Terminology Criteria for Adverse Events Version 5.0[14] – absolute neutrophil count < 1000/mm^3 with a single temperature of > 38.3˚C or a sustained temperature of ≥ 38˚C for more than one hour – and treatment discontinuation), and the number need to screen (NNS) - referring to the number of patients that need to be screened with 21-gene RS assay to prevent one CRAE. We also evaluated the feasibility of this genomic assay protocol.

As exploratory analyses we 1) interpreted the RS results according to the subsequently published TAILORx/RxPONDER data,[5,6] 2) measured the association of RS with histologic subtype and grade, and 3) tested the correlation of RS and Ki67 for the whole cohort and for the node-negative patients from inclusion criterion iii).

Statistical analysis

We performed a descriptive analysis using median and range for quantitative variables and absolute and relative frequencies for categorical variables. The cumulative incidence of adjuvant chemotherapy de-escalation was assessed in the whole cohort and by inclusion criteria subgroup. The 95% confidence intervals (95% CI) for proportions were estimated using the binomial 'exact' method.[15] The NNS was calculated using the formula NNS = NNT/prevalence,[16] with NNT representing the number of patients that need to be de-escalated to prevent one patient from having at least one CRAE (NNT = 1/ARR). Calculations considered the absolute risk reduction (ARR) in the proportion of patients with at least one CRAE resulting from de-escalating adjuvant chemoendocrine therapy to isolated endocrine therapy due to RS < 18, and the prevalence of RS < 18 observed in our sample. The associations of histologic subtype and grade with RS cut-offs (≥ 18 *vs* < 18, and > 25 *vs* ≤ 25) were evaluated with the chi-squared test or Fisher's exact test as appropriate, and the correlation between Ki67 and RS was tested with Pearson correlation coefficient. We used R and significance level of 5%.

RESULTS

Cohort characteristics

We included 154 women, 23 (15%) by criterion i) luminal A-like pT2pN0, seven (5%) by criterion ii) luminal A-like, 1 – 3 positive nodes, with comorbidities/PS that confer higher risk for chemotherapy-induced toxicity, and 110 (71%) by criterion iii) pT1-2pN0, PR ≤ 20% and/or Ki67 14% – 40%. Fourteen patients (9%) were included with more than one criterion [Appendix 1, S1 (Appendix 1: https://www.actamedicaportuguesa.com/revista/index.php/amp/article/view/18539/15038)].

The median age was 61 years (range, 25 – 79), 69% were postmenopausal, and tumours were mostly pT1 (55%), pN0 (82%), invasive ductal (73%), grade 2 (86%), luminal B-like (69%), stage IA (85%), and with a median Ki67 of 20% (1% – 40%). Surgery was lumpectomy plus sentinel lymph node biopsy in 104 cases (67%), mastectomy plus sentinel lymph node biopsy in 31 (20%), mastectomy plus axillary lymph node dissection in 15 (10%) and lumpectomy with axillary lymph node dissection in four (3%) (Table 1).

Recurrence score and impact on the adjuvant treatment decision

We obtained RS < 18 in 60% (95% CI: 52% – 68%; 93/154), ≥ 18 in 38% (30% – 47%, 59/154) and undetermined in 1% (0.2% – 5.0%, 2/154). In patients with luminal A-like, pT2pN0 tumours (n = 23) only two had RS ≥ 18 (RS = 19 and 20). From luminal A-like node-positive patients included due to comorbidities or PS that conferred higher risk for chemotherapy-induced toxicity (n = 7), two had RS ≥ 18 (RS = 19 and 20). Regarding patients included by PR ≤ 20% and/or Ki67 14% – 40% (n = 110), 49 (45%) presented RS ≥ 18. Out of the 14 included with more than one criterion, six patients (43%) had a RS ≥ 18 (Fig. 1).

We found an overall adjuvant treatment de-escalation of 65% (57% – 72%, 100/154), 58% (50% – 66%, 90/154) per protocol (Table 2). Patients with RS < 18 (n = 93), as per protocol, were discussed for treatment de-escalation for isolated adjuvant endocrine therapy with or without

Table 1 – Cohort baseline characteristics

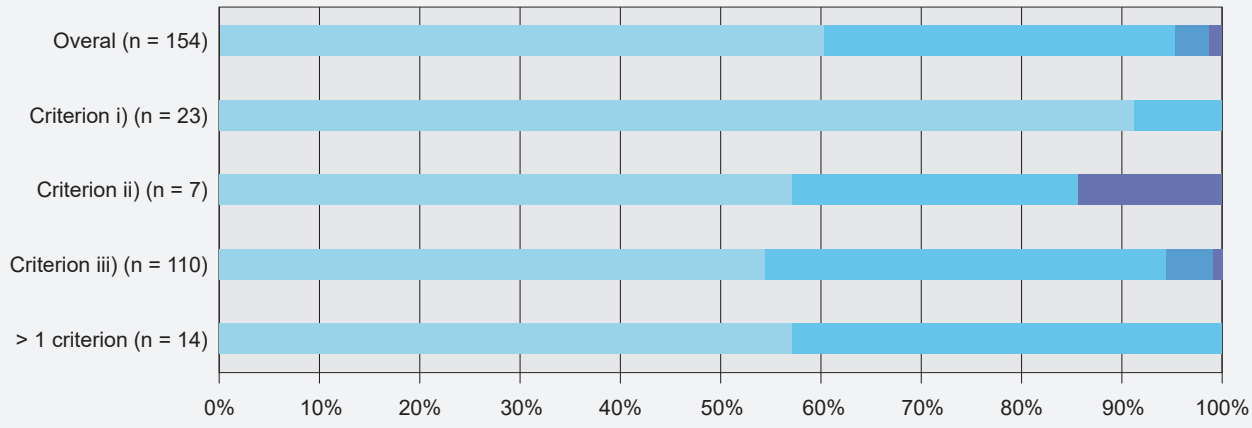
		n = 154
Age	Median (range)	61 (25 – 79)
	≤ 50 years (%)	43 (28)
Menopausal status, n (%)	Pre-	48 (31)
	Post-	106 (69)
Surgery, n (%)	Lumpectomy + SLNB	104 (67)
	Lumpectomy + ALND	4 (3)
	Mastectomy + SLNB	31 (20)
	Mastectomy + ALND	15 (10)
pT, n (%)	1	85 (55)
	2	69 (45)
pN, n (%)	0	126 (82)
	(1 – 3gg)	28 (18)
Histologic subtype, n (%)	Invasive ductal carcinoma	112 (73)
	Invasive lobular carcinoma	20 (13)
	Invasive carcinoma with ductal and lobular features	10 (6)
	Mixed ductal and mucinous carcinoma	8 (5)
	Carcinoma with invasive papillary component	2 (1)
	Carcinoma with neuroendocrine component	1 (< 1)
	Tubular/cribriform carcinoma	1 (< 1)
Histologic grade, n (%)	1	18 (12)
	2	132 (86)
	3	4 (3)
Luminal, n (%)	A-like	48 (31)
	B-like	106 (69)
Ki67, median (range)		20 (1 – 40)
AJCC/TNM staging, 8th edition, n (%)	IA	131 (85)
	IB	12 (8)
	IIA	10 (6)
	IIB	1 (< 1)

ALND: axillary lymph node dissection; SLNB: sentinel lymph node biopsy

adjuvant radiotherapy. However, three patients (3%) were proposed for adjuvant chemoendocrine therapy. The remaining 90 patients (97%) were proposed for isolated adjuvant endocrine therapy as per protocol, mostly with aromatase inhibitor (62%, 56/90). Of patients with RS ≥ 18 (n = 59), 51 patients (86%) were proposed for adjuvant chemotherapy as per protocol, mostly with a taxane-based regimen (63%, 32/51). Eight patients with RS ≥ 18 (14%, 8/59), were reconsidered for isolated endocrine therapy. The two patients with an undetermined RS result were proposed for isolated endocrine therapy due to a delay longer than three months from surgery date, after two attempts of genomic assay (Table 2).

Cohort follow-up: clinical outcomes

With a median follow-up of 51 months (range: 25 – 77), there were two local (1%) and six distant recurrences (4%). The local recurrences occurred 33 months after lumpectomy and sentinel lymph node biopsy in a patient with RS = 7 who refused to receive adjuvant radiotherapy and tamoxifen, and 25 months after lumpectomy and sentinel lymph node biopsy in a premenopausal, node-negative woman with RS = 19 who suspended adjuvant chemotherapy after a hypersensitivity reaction to taxane during the first cycle. The distant recurrences occurred in women with RS = 10, 14, 25, 28, 29, and 44, with a median of 31 months (18 – 38) until the distant recurrences observed. Except from the postmenopausal node-negative patient with RS = 25,



	> 1 criterion (n = 14)	Criterion iii) (n = 110)	Criterion ii) (n = 7)	Criterion i) (n = 23)	Overal (n = 154)
RS < 18	8	60	4	21	93
RS 18 – 30	6	44	2	2	54
RS ≥ 31	0	5	0	0	5
RS und.	0	1	1	0	2

RS < 18 RS 18 – 30 RS ≥ 31 RS und.

Figure 1 – Recurrence score (RS) distribution in the whole cohort and by institutional 21-gene assay protocol inclusion criterion. Inclusion criteria: i) Luminal A-like, pT2pN0; ii) Luminal A-like, with 1 – 3 involved axillary nodes and presence of comorbidities or performance status that constitute a higher risk for chemotherapy-induced toxicity; iii) pT1-2pN0, PR ≤ 20% and/or Ki67 14% – 25%.

who received isolated endocrine therapy after TAILORx data publication,[5] all the other patients received adjuvant chemotherapy. The woman with RS = 10, was proposed for adjuvant chemoendocrine therapy due to a diagnosis of supraclavicular node-positive disease after initial staging, surgery, and genomic assay, while the woman with RS = 14 was given adjuvant chemoendocrine therapy by patient-clinician joint decision. There was one case of contralateral breast cancer in a postmenopausal women treated with isolated endocrine therapy after a RS = 12 (1%). There were five deaths (3%), two due to breast cancer progressive disease and three from a non-breast cancer cause. Regarding CRAE during the first six months of adjuvant therapy, chemotherapy was associated with higher rate of patients with unscheduled medical visits (31%, 17/54 *vs* 5%, 5/100). From patients who attended unscheduled medical visits, those receiving adjuvant chemotherapy showed a trend for more visits (median 2, range 1 – 6 *vs* 1, 1 – 1). Patients receiving isolated endocrine therapy did not have any hospital admission during the first six months of adjuvant treatment, while 13% (7/54) of patients receiving chemotherapy experienced a hospital admission with a median duration of eight days (range: 1 – 18). The rate of patients with grade 3 febrile neutropenia under chemotherapy was 17% (9/54). Chemotherapy was discontinued due to docetaxel adverse events in 9% (5/54), in two of them due to infusion reactions. Overall, 35% (19/54) of the patients treated with chemotherapy experienced at least one CRAE, compared with 5% (5/100) of those treated with isolated endocrine therapy, which represents an absolute risk reduction of 30% (95% CI: 17% – 44%) [Appendix 1, S2 (Appendix 1: https://www.actamedicaportuguesa.com/revista/index.php/amp/article/view/18539/15038)].

Considering the 30% absolute risk reduction of CRAE observed in the de-escalated patients treated with isolated endocrine therapy, and that by our sample inclusion criteria 60% of tested patients would be de-escalated to isolated endocrine therapy as a result of RS < 18, seven (95% CI: 5 – 10) patients needed to be screened with the 21-gene RS assay to prevent one CRAE during the first six months of adjuvant treatment.

Protocol feasibility

There was an increase in the number of tests requested per trimester across the study period, mainly after revision of the protocol criteria in April 2017. This number ranged from five (February – April 2016 - third trimester of the protocol) to 23 (February – April 2018 - second last trimester of the protocol) [Appendix 1, S3 (Appendix 1: https://www.actamedicaportuguesa.com/revista/index.php/amp/article/view/18539/15038)].

Regarding the compliance with inclusion criteria, we

found 15 protocol deviations (10%) with the inclusion of node-positive patients by criterion iii). The median time from sample shipment to the central laboratory to 21-gene RS assay feedback was eight (range: 3 – 27) days. The proportion of cases that needed to repeat the assay was 4% (6/154), and the result was undetermined due to insufficient sample in 1% (2/154). As described above and shown in Table 2, there were 11 protocol deviations (7%) concerning the multidisciplinary team meeting proposal for adjuvant treatment.

Interpretation of RS according to TAILORx/RxPONDER data

From the 126 node-negative patients tested, 38% (48/126) had a RS ≥ 18, but only 9% (11/126) had RS > 25 [Appendix 1, S4A (Appendix 1: https://www.actamedicaportuguesa.com/revista/index.php/amp/article/view/18539/15038)] and all of them were given adjuvant chemoendocrine therapy. Within the 79 patients aged > 50 years with RS 0 – 25, 32% (25/79) had a RS 18 – 25 and of these 80% (20/25) were given chemoendocrine therapy as per protocol. On the other hand, from the 19 patients aged ≤ 50 years with RS > 15, 32% (6/19) had a RS 16 – 17 and were given isolated endocrine therapy as per protocol. Considering our protocol inclusion criteria, only criterion i) – luminal A-like pT2pN0 – did not register any case of RS > 25 (S4A). However, within this subgroup there were two out of ten patients aged ≤ 50 years who had a RS > 15 (S4C). From the 28 node-positive patients tested, 39% (11/28) had RS ≥ 18, but only 11% (3/28) had RS > 25. The three were postmenopausal and were given adjuvant chemoendocrine therapy. Among the 20 postmenopausal patients with RS 0 – 25, 25% (5/20) had a RS 18 – 25, and of these, four were given adjuvant chemoendocrine therapy, as per protocol. Four out of the eight premenopausal patients were given isolated endocrine therapy [Appendix 1, S5 (Appendix 1: https://www.actamedicaportuguesa.com/revista/index.php/amp/article/view/18539/15038)]. According to the current

Table 2 – Multidisciplinary team (MDT) proposal and adjuvant treatment starting choice

Recurrence Score	MDT proposal	Adjuvant treatment - starting choice	
Low (< 18) (n = 93)	Endocrine therapy (n = 90) – per protocol	Aromatase inhibitor (n = 56)	Letrozole (n = 56)
		Tamoxifen (n = 34)	
	Chemoendocrine therapy (n = 3)	Anthracycline and taxane based (n = 2)	FEC3 + paclitaxel (n = 2)
		Taxane-based (n = 1)	TC4 (n = 1)
Intermediate/high (≥ 18) (n = 59)	Chemoendocrine therapy (n = 51) – per protocol	Taxane-based (n = 32)	TC4 (n = 31)
			TC6 (n = 1)
		Anthracycline and taxane based (n = 16)	FEC-D (n = 10)
			FEC3 + paclitaxel (n = 6)
		Anthracycline based (n = 3)	FEC6 (n = 2)
			AC4 (n = 1)
	Endocrine therapy (n = 8)	Aromatase inhibitor (n = 7)	Letrozole (n = 6)
			Exemestane (n = 1)
		Tamoxifen (n = 1)	
Undetermined (n = 2)	Endocrine therapy (n = 2)	Aromatase inhibitor (n = 2)	Letrozole (n = 2)

AC4: four cycles, three weekly, of doxorubicin 60 mg/m² IV and cyclophosphamide 600 mg/m² IV; FEC3: three cycles, three weekly, of fluorouracil 500 mg/m² IV, epirubicin 100 mg/m² IV and cyclophosphamide 500 mg/m² IV – two patients were given reduced dose of epirubicin – 75 mg/m² IV per cycle; FEC6: six cycles, three weekly, of fluorouracil 500 mg/m² IV, epirubicin 100 mg/m² IV and cyclophosphamide 500 mg/m² IV; FEC-D: FEC3 followed by three cycles, three weekly, of docetaxel 100 mg/m² IV; Paclitaxel: nine to 12 cycles, weekly, 80 mg/m² IV; TC4: four cycles, three weekly, of docetaxel 75 mg/m² IV and cyclophosphamide 600 mg/m² IV; TC6: six cycles, three weekly, of docetaxel 75 mg/m² IV and cyclophosphamide 600 mg/m² IV

guidance for tailoring adjuvant treatment, the inclusion criteria of this institutional protocol identified 76% (96/126) of the node-negative patients and 57% (16/28) of node-positive patients with a RS compatible with adjuvant treatment de-escalation and with indication for treatment with isolated endocrine therapy. This would mean an overall treatment de-escalation of 73% (112/154) with the currently used RS cut-offs.

Relationship between RS and histology or Ki67

There were no associations between RS categories and histologic subtype or grade [Appendix 1, S6 (Appendix 1: https://www.actamedicaportuguesa.com/revista/index.php/amp/article/view/18539/15038)]. There was no RS > 25 for grade 1 tumours (n = 18), but within the six cases of grade 1 tumours with RS > 15, five were aged ≤ 50 years, and three were node-positive with RS ≥ 18 (RS 18 and 22).

There was a statistically significant weak correlation (r = 0.22, 95% CI: 0.07 – 0.37) between Ki67 and RS for the whole cohort, but no correlation could be demonstrated for the subgroup of node-negative patients from inclusion criterion iii) [Appendix 1, S7 (Appendix 1: https://www.actamedicaportuguesa.com/revista/index.php/amp/article/view/18539/15038)].

DISCUSSION

This study found that the use of the selected clinicopathologic inclusion criteria for 21-gene RS assay was associated with decreased chemotherapy exposure in at least six out of each 10 tested women. This protocol avoided unnecessary treatment toxicity by preventing one clinically relevant adverse event by every seven women that undergo testing. Another important finding was that all women older than 50 years and included in our study due to pT2pN0 tumours with PR > 20% and Ki67 < 14% presented a RS ≤ 25. Although stronger evidence should be used to support this decision, this finding suggests that this latter subgroup could be eventually spared from adjuvant chemotherapy without the use of this genomic assay.

An accurate selection of patients with uncertain benefit from adjuvant chemotherapy, but a higher likelihood of low RS, might be the key to increase the cost-effectiveness of this tool that has already been demonstrated in other countries.[17–20] This was the reason to select more restrictive inclusion criteria than those considered eligible for genomic assay: HR+/HER2-, pT1 – 2, 0 – 3 nodes.[21] Therefore, we did not include patients with pT1pN0 tumours with PR > 20% and Ki67 below 14%, for whom local and international guidelines do not recommend adjuvant chemotherapy.[22,23] On the other hand, we also did not include patients with higher-risk tumours who have indication to receive adjuvant chemotherapy, such as luminal B-like node-negative with Ki67 > 40% or luminal B-like with 1 – 3 positive nodes.[22,23] By excluding these cases, the eligibility was restricted to women with tumours belonging to a shorter 'grey area' of risk, to whom adjuvant chemotherapy would have being prescribed as per local standards. This resulted in a higher proportion of treatment de-escalation than those reported in real-word studies from other European countries.[24,25]

Considering the results from the TAILORx trial, that prospectively validated the increase of the cut-off for adjuvant chemotherapy in node-negative disease to RS > 25,[5] the reported percentage of patients spared from adjuvant chemotherapy with this protocol would be even higher (76%), even though still slightly above than what was more recently reported in another European country.[26] Twenty additional women could have been de-escalated for isolated adjuvant endocrine therapy, while only six women with ≤ 50 years with a RS 16 – 17 could have had benefitted from adjuvant chemotherapy.

Regarding node-positive disease and considering the new data from the RxPONDER trial,[6] we should consider that all premenopausal women with node-positive disease may benefit from adjuvant chemotherapy, regardless of RS. Thus, in premenopausal women, the use of OncotypeDx should be restricted to node-negative patients. However, the selection of node-positive postmenopausal women in this cohort was compatible with a high de-escalation proportion (80%), with only three cases of RS > 25. Therefore, the use of this genomic assay should be extended to more postmenopausal women with luminal A-like tumours and 1 – 3 positive nodes.

Our study did not find a significant association of RS with either histologic subtype or grade, as suggested by larger studies.[8,9] Indeed, our data suggests that selected well-differentiated tumours in women with aged under 50 years old, or node-positive disease might still benefit from the genomic assay. Despite the weak correlation in the whole cohort, we found no correlation between Ki67 and RS in patients with node-negative and Ki67 14% – 40%. Therefore, our data do not allow drawing any conclusions on to what extent histology features or Ki67 could be used to redefine eligibility criteria for the use of this genomic assay. These uncertainties reinforce the utility of this tool in tailoring the adjuvant treatment.

Importantly, the use of this genomic assay protocol did not compromise the adequate timing for adjuvant chemotherapy, with low proportion of undetermined RS. We reported an increasing number of tests per trimester as clinicians were recognising the clinical usefulness of this tool, which, along with the pre-defined restrictive inclusion criteria, might explain the inclusion of only 9% of all stage I-II HR+/HER2- breast cancer cases treated in the institution during the period of the study.

This study has some methodological limitations, such as using data from a single-centre and employing an observational design, which limit both external and internal validity of the results. Moreover, the sample size limits the statistical power to explore associations or correlations between RS and clinicopathologic features. The 10% rate of eligibility protocol deviations, derived from the inclusion of node-positive patients with more aggressive tumours than permitted by the protocol, might have generated bias towards a lower overall rate of adjuvant treatment de-escalation. On the other hand, the 7% rate of protocol deviations related to adjuvant treatment recommendations was in part influenced by the shift in the cut-off for adjuvant chemotherapy after the publication of the results from the TAILORx trial,[5] and did not lead to disease recurrence due to potential undertreatment. Despite these limitations, and to the best of our knowledge, this is the first report of a wide institutional protocol use of this 21-gene RS assay in a Portuguese public hospital, providing guidance on how to potentiate the use of a limited resource in this setting.

CONCLUSION

In the era of personalised medicine, as genomic assays are becoming widespread in developed countries, it is important that clinicians can recognise which patients may benefit the most from them. This study identified inclusion criteria for performing genomic assay in women with HR+/HER2- early breast cancer leading to de-escalation of adjuvant systemic treatment in at least six out of ten women undergoing testing, and prevention of one clinically relevant adverse event in one out of seven women undergoing testing. This assay is replicable in real-world settings and does not considerably delay the appropriate timing for adjuvant systemic treatment.

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PRESENTATIONS AND AWARDS

Preliminary findings were presented and awarded with the best oral communication at the *Encontros da Primavera 2017*, and the final cohort results were presented in 2018 at the *X Congresso Nacional de Senologia*, both national meetings.

DATA AVAILABILITY STATEMENT

All data generated or analyzed during this study are included in this article and its supplementary material files. Further enquiries can be directed to the corresponding author.

AUTHOR CONTRIBUTIONS

DMB: Data curation; formal analysis; investigation; methodology; project administration; resources; validation; visualization; writing of the draft.

SCF: Data curation; methodology; validation; visualization; writing of the draft; manuscript review and editing.

EG: Conceptualization; investigation; methodology; resources; validation; visualization; manuscript review and editing.

SA: Conceptualization; data curation; investigation; methodology; resources; validation; visualization; manuscript review and editing.

SE: Formal analysis; methodology; resources; validation; visualization; manuscript review and editing.

MB: Conceptualization; data curation; investigation; methodology; project administration; resources; validation; visualization; manuscript review and editing.

AM: Conceptualization; investigation; methodology; project administration; resources; supervision; validation; visualization; manuscript review and editing.

PROTECTION OF HUMANS AND ANIMALS

The authors declare that the procedures were followed according to the regulations established by the Clinical Research and Ethics Committee and to the Helsinki Declaration of the World Medical Association updated in 2013.

DATA CONFIDENTIALITY

The authors declare that they followed the protocols in use at their working center regarding patients' data publication.

COMPETING INTERESTS

DMB declares honoraria from Daiichi Sankyo, Novartis, Merck Sharp & Dohme, Janssen, Pfizer, Angelini, and AstraZeneca; meeting/travel grants from Novartis, Merck Sharp & Dohme, LEO Farmacêuticos, Ipsen, and Janssen; and institutional grants from Novartis and F. Hoffmann-La Roche Ltd (all outside the submitted work).

SCF declares honoraria from Novartis, and travel grants from Pfizer and Roche (all outside the submitted work). EG declares honoraria from Pierre Fabre, Merck Sharp & Dohme, Pfizer, Bristol-Myers Squibb, Novartis, Incyte Biosciences Iberia, Grunenthal and Roche, and meeting/travel grants from Merck Sharp & Dohme, Bristol-Myers Squibb, Roche, Pierre Fabre and PharmaMar (all outside the submitted work).

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COVID-19-associated Coagulopathy Characterization using Rotational Thromboelastometry in a Prospective, Observational Cohort Study: The HemoCoV Study

Caracterização da Coagulopatia Associada ao COVID-19 usando Tromboelastometria Rotacional num Estudo Observacional de Coorte Prospetivo: Estudo HemoCov

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ABSTRACT

Introduction: COVID-19-associated coagulopathy includes systemic and endothelial inflammation with coagulation dysregulation related to immunothrombosis. The aim of this study was to characterize this complication of SARS-CoV-2 infection in patients with moderate to severe COVID-19.

Methods: An open-label, prospective observational study conducted in patients with COVID-19 moderate to severe acute respiratory failure admitted to an intensive care unit (ICU). Coagulation testing, including thromboelastometry, biochemical analysis and clinical variables, were collected at pre-specified time points during the 30 days of ICU stay.

Results: The study included 145 patients, 73.8% male, with a median age of 68 years (interquartile range - IQR 55 - 74). The most prevalent comorbidities were arterial hypertension (63.4%), obesity (44.1%) and diabetes (22.1%). Simplified acute physiology score II (SAPS II) was on average 43.5 (11 - 105) and sequential organ failure assessment (SOFA) at admission was 7.5 (0 - 14). During ICU stay, 66.9% of patients underwent invasive mechanical ventilation and 18.4% extracorporeal membrane oxygenation support; thrombotic and hemorrhagic events occurred in 22.1% and 15.1% of the patients respectively; anticoagulation with heparin was present in 99.2% of patients since early ICU stay. Death occurred in 35% of patients. Longitudinal studies revealed changes in almost all coagulation tests during the ICU stay. SOFA score, lymphocyte counts, some biochemical, inflammatory and coagulation parameters, including hypercoagulability and hypofibrinolysis seen in thromboelastometry, differed significantly ($p < 0.05$), between ICU admission and discharge. Hypercoagulability and hypofibrinolysis persisted throughout ICU hospitalization, showing higher incidence and severity in non-survivors.

Conclusion: COVID-19-associated coagulopathy is characterized by hypercoagulability and hypofibrinolysis from ICU admission, and persisted throughout the clinical course in severe COVID-19. These changes were more pronounced in patients with higher disease burden and in non-survivors.

Keywords: Blood Coagulation Disorders; COVID-19; Fibrinolysis; Thromboelastometry; Thrombosis

RESUMO

Introdução: A coagulopatia associada à COVID-19 inclui inflamação sistémica e endotelial com desregulação da coagulação relacionada com imunotrombose. O objetivo deste estudo foi caracterizar esta complicação da infecção por SARS-CoV-2 em doentes com infeção COVID-19 moderada a grave.

Métodos: Estudo prospetivo observacional *open-label* conduzido em doentes com insuficiência respiratória aguda COVID-19 moderada a grave admitidos numa unidade de cuidados intensivos (UCI). Testes da coagulação, incluindo tromboelastometria, testes de bioquímica e variáveis clínicas foram colhidos em pontos de análise predefinidos durante 30 dias de internamento na UCI.

Resultados: Foram incluídos 145 doentes, 73,8% homens, com uma mediana de idade de 68 anos (intervalo interquartílico – IIQ 55 - 74). As comorbilidades mais prevalentes foram hipertensão arterial (63,4%), obesidade (44,1%) e diabetes (22,1%). Na admissão, o *simplified acute physiology score II* (SAPS II) apresentou uma mediana de 43,5 (11 - 105) e o *sequential organ failure assessment* (SOFA) de 7,5 (0 - 14). Durante a estadia na UCI, 66,9% dos doentes foram submetidos a ventilação mecânica invasiva e 18,4% a suporte com *extracorporeal membrane oxygenation*; Eventos trombóticos e hemorrágicos ocorreram em 22,1% e 15,1% dos doentes respetivamente; anticoagulação com heparina esteve presente em 99,2% dos doentes desde precocemente durante a estadia na UCI. A morte ocorreu em 35% dos doentes. Estudos longitudinais revelaram alterações em quase todos os testes da coagulação durante a hospitalização na UCI. O SOFA *score*, a contagem de linfócitos, alguns parâmetros bioquímicos, inflamatórios e da coagulação, incluindo hipercoagulabilidade e hipofibrinólise observados na tromboelastometria, diferiram significativamente ($p < 0,05$), entre a admissão e a alta da UCI. A hipercoagulabilidade e a hipofibrinólise persistiram ao longo da hospitalização na ICU, mostrando maior incidência e gravidade nos doentes não sobreviventes.

Conclusão: A coagulopatia associada à COVID-19 é caracterizada por hipercoagulabilidade e hipofibrinólise desde a admissão na UCI, as quais persistiram durante o curso clínico na infeção COVID-19 grave. Estas alterações foram mais pronunciadas nos doentes com maior gravidade e nos não sobreviventes.

Palavras-chave: COVID-19; Fibrinólise; Perturbações da Coagulação Sanguínea; Tromboelastometria; Trombose

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INTRODUCTION

SARS-CoV-2 infection has been associated with a syndrome dominated by acute respiratory failure (ARF). However, the pathophysiology of this infection goes well beyond severe hypoxemia, its most feared manifestation. Immunological activation and coagulation dysregulation have been recognized as important mechanisms leading to thrombus-inflammation, thrombosis[1-3] and endothelitis.[1]

COVID-19 associated coagulopathy (CAC) is marked by profound hyperinflammation with liberation of inflammatory mediators,[4] endothelial dysfunction and injury, abnormal blood flow dynamics, platelet and coagulation factor activation, all of them leading to hypercoagulability and systemic fibrinolysis shutdown.[5,6] Moreover, real life evidence in severe COVID-19 shows hyperinflammation, T cell deficiencies and coagulation abnormalities associated with life-threatening organ dysfunction.[7] These complex chains of events induce a state of immunothrombosis leading to microvascular thrombosis.[1,4-6,8] The recognition of this prothrombotic state might explain why some laboratory parameters were viewed as potential surrogate markers of poor outcomes.[9-13] Dynamic cytokine storms and T cell lymphopenia are associated with COVID-19 severity as well,[7,14,15] making lymphocyte count another possible marker to identify patients at risk of developing severe COVID-19.[14]

Current International guidelines recommend prophylactic anticoagulation with heparin in all COVID-19 hospitalized patients, which should be tailored in selected patients.[16-21]

Assuming that coagulation abnormalities are paramount to define the outcome in COVID-19 patients, we designed a single-center study involving critically ill patients with SARS-CoV-2 infection and severe respiratory failure. We hypothesized that extensive CAC characterization may identify factors related to disease severity, contributing to a better understanding of COVID-19 pathophysiology.

METHODS

Study design and setting

The present study is part of a major research project named HemoCoV: an open-label, real-life, prospective non-interventional, cohort study, conducted by the Transfusion Medicine and the Intensive Care Departments of an Academic Tertiary Care Hospital Centre.

The HemoCoV study was approved by the Academic Hospital Centre Ethics Committee (reference number 295/20). STROBE recommendations for cohort studies were followed.[22]

Participants

Consecutive adult patients admitted to the Intensive Care Department (ICU) with a diagnosis of COVID-19-related acute respiratory failure (ARF) were evaluated for enrollment between August 20, 2020 and January 15, 2021.

Inclusion criteria were assessed and written informed consent was obtained in all patients.

The diagnosis of SARS-CoV-2 infection was confirmed by two positive polymerase chain reaction tests, in agreement with recommendations from national health authorities. Respiratory failure was defined as oxygen requirement administered by either high-flow nasal cannula, non-invasive ventilation or invasive mechanical ventilation (IMV), consistent with the World Health Organization (WHO) clinical progression scale definition of hospitalized severe disease.[23]

Inclusion and exclusion criteria

Inclusion criteria were patients aged 18 years or older with confirmed SARS-CoV-2 infection admitted to the ICU with moderate to severe hypoxemia (PaO_2/FiO_2 ratio below 200), irrespective of ventilatory status.

The exclusion criteria included pregnancy, previously diagnosed cognitive disorder (preventing informed consent), negative or SARS-CoV2 test not performed, absence of blood sample for thromboelastometry analysis within the first 24 hours of ICU admission, patients presenting with septic shock, severe liver failure, active cancer disease, history of congenital coagulation disorders, surgical procedures in the last four weeks, death before 24 hours of ICU admission, and withdrawal, refusal or inability to sign the informed consent form.

Sample size

In this study, 151 patients were included between August 2020 and January 2021. Six patients were withdrawn from the analysis for meeting the exclusion criteria. The reasons for exclusion were active cancer (n = 1), missing data (n = 3), death in the first 24 hours (n = 1) and SARS-CoV-2 infection not confirmed (n = 1).

Outcomes

The primary outcome was extensive CAC characterization at ICU admission and comparison with ICU discharge data.

The secondary outcomes were extensive CAC characterization at predefined time-points until day 30 of ICU stay or until ICU discharge and comparison of thromboelastometric parameters between survivors and non-survivors.

Intervention

Blood samples for AB0 blood type, complete blood count (CBC), thromboelastometry (ROTEM®), coagulation tests [D- dimer, prothrombin time–PT, activated partial thromboplastin time-aPTT, fibrinogen, factor (F) VIII, von Willebrand

factor (vWF): antigen (Ag) and ristocetin cofactor (RCo), antithrombin], interleukin-6 (IL-6), ferritin, C-reactive protein (CRP), procalcitonin (PCT), renal and liver function tests and blood gas (including ionized calcium-iCa^{2+}) were collected in the following time points: within 24 hours after ICU admission; immediately after implementing IMV or extracorporeal membrane oxygenation (ECMO); immediately after a diagnosis of a thrombotic or hemorrhagic event; every five-day interval along the initial 30 days of ICU admission; at ICU discharge. Blood samples were not reassessed if two events happened within four hours.

A clinically significant hemorrhagic event was defined as grade 2 or higher of the WHO bleeding score.[24] A clinically significant thrombotic event was defined as grade 2 or higher of the National Cancer Institute - Common Terminology Criteria for Adverse Events.[25] Glomerular Filtration Rate (GFR) was defined by the Chronic Kidney Disease Epidemiology Collaboration formula.[26,27] Computed tomographic angiography of the lung was performed in patients with clinical suspicion of lung microthrombosis or a major thromboembolic event. Doppler was done if there was clinical suspicion of a thrombotic event, and/or at ICU discharge. The hypercoagulability profile was defined by thromboelastometry parameters as clotting time (CT)-EXTEM < 45 seconds,[28] clot formation time (CFT)-EXTEM < 50 seconds,[29] maximum clot firmness (MCF)-EXTEM > 68 mm,[28,29] MCF-FIBTEM > 22 mm[28]; and hypofibrinolysis as lysis index at 60 minutes after CT (LI60)-EXTEM ≥ 97%,[28,29] lysis index at 30 minutes after CT (LI30) ≥ 97%, LI60 ≥ 97%, and maximum lysis (ML) < 5% in any thromboelastometry assay.

Additional assessments at specific time points (implementation of IMV and ECMO, diagnosis of thrombotic or hemorrhagic events) will be left for further evaluations.

Clinical data

Patient baseline characteristics were registered, including blood group, relevant comorbidities and previous antithrombotic therapy. Risk and prognostic scores were also evaluated [simplified acute physiology score II (SAPS II), sequential organ failure assessment (SOFA), sepsis-induced coagulopathy score SIC)[30] and disseminated intravascular coagulopathy score (DIC)].[31]

Clinical data were prospectively collected, including ventilatory mode, plateau pressure, driving pressure, $EtCO_2$/$PaCO_2$ ratio, and PaO_2/FiO_2 ratio. Anticoagulation therapy during ICU hospitalization was also registered. Hemorrhagic and thrombotic events were classified as complications, and triggered evaluation of coagulation parameters according to the national guidelines and as defined previously in this study, but will be left for further evaluation. Survival was defined by hospital discharge, either to the community or to another referring hospital. Mortality was defined as occurrence of death during the whole in-hospital stay, which includes ICU or subsequent ward admissions.

Laboratorial data

All laboratory assays were performed according to the standardized manufacturer protocols, comprising: CBC parameters (Coulter DX4900, Beckman-Coulter, California, USA); PT, aPTT, fibrinogen, FVIII, vWF:Ag, vWF:RCo, antithrombin (ACL TOP750, Werfen, Barcelona, Spain); D-dimer (ACL TOP750 CT5, Werfen, Barcelona, Spain); IL-6, ferritin, CRP, PCT, lactate dehydrogenase (LDH), creatinine, urea, aspartate aminotransferase (AST), alanine aminotransferase (ALT), albumin, bilirubin, and troponin (Cobas 8000, Roche, Basel, Switzerland), and iCa^{2+} by blood gas analysis.

Thromboelastometry (ROTEM®)

Rotational thromboelastometry is a viscoelastic testing (VET) system, which evaluates the global coagulation process on whole blood.[28] VET allows for a global assessment of clot initiation (e.g., CT), clot strength or amplitude (amplitude at five minutes after CT-A5, amplitude at 10 minutes after CT-A10, MCF- e.g. fibrinogen and platelet contribution), and clot stability (e.g. fibrinolysis: LI30, LI60, ML).[32] In order to evaluate fibrinolysis, the established run time was 70 minutes to achieve ML and LI60. Four assays assessing extrinsic (EXTEM) and intrinsic (INTEM) clot activation, fibrinogen contribution to clot formation (FIBTEM), and heparin presence (HEPTEM) were done at the same time and at different time points of the previously described study analysis, using a ROTEM® Delta device (Werfen, Barcelona, Spain), and following the manufacturer`s instructions.

Statistical methods

Baseline clinical characteristics, as well as clinical and laboratory data, collected at the specified time points were compared between survivors and non-survivors.

Patient characteristics are presented as median, with the respective interquartile range (IQR) for the continuous variables, according to the distribution underlying the data, and as number (n) and percentage (%) for the categorical variables. The normality underlying the data was evaluated using the Shapiro-Wilk test.

Statistical comparisons between two independent groups were performed with the Mann–Whitney U test for continuous variables and with the χ^2 test for categorical variables or Fisher's exact test when applicable. Regarding paired data, statistical comparisons were performed using the Wilcoxon test.

All the results with a p-value < 0.05 were considered significant. The statistical analysis was performed using the software R Studio version 4.1.2. In case of missing data for

a specific variable, these patients were not included in the analysis including this same variable. No imputation was performed.

RESULTS

Sample characteristics

A total of 151 consecutive patients were included. Of these, six patients were excluded. The remaining 145 patients were eligible and were included in the analysis. The patient's main characteristics at ICU admission and during ICU hospitalization are described in Tables 1 and 2, respectively.

Patients included in our study were predominantly white and male (86.9% and 73.8%, respectively) with a median age of 64 years (IQR 55 - 74) (Table 1). Arterial hypertension was the most prevalent comorbidity (63.4%). Previous thrombosis was documented in six patients. At ICU admission, 95 patients were already under anticoagulant therapy, mainly prophylactic low molecular weight heparin (LMWH).

During ICU hospitalization (Table 2), 66.9% required IMV, and 18.4% were treated with ECMO support. A thrombotic or hemorrhagic event occurred in 22.1% and 15.1% of patients, respectively. From early on during the ICU stay, 99.2% of patients received anticoagulation, mostly with prophylactic and intermediate doses of LMWH. Overall, a mortality rate of 35% was observed.

Clinical characteristics

Appendix 1 (Appendix 1: https://www.actamedicaportuguesa.com/revista/index.php/amp/article/view/19475/15148) shows the evolution of different laboratory parameters plus clinical scores at different time points of the analysis during the 30 days of ICU stay. The main parameters with a significant *p*-value ($p < 0.05$) evaluated

Table 1 – Patient characteristics at Intensive Care Unit admission

Parameter	n (%)	IQR
Total number of patients	145	
White	126 (86.9)	
Male	107 (73.8)	
Age (median) (years)	64	55 - 74
Weight (median) (kg)	82	73 - 96
Symptom-to-hospital admission (median)(days)	7	5 - 10
Symptom-to-ICU admission (median)(days)	9	7 - 12
Blood groups:		
Group O:	52 (35.9)	
Group non-O:	87 (60)	Non-O: A:63; B:17;AB:7
Unclassified:	6 (4.1)	
Comorbidities:		
Arterial hypertension	92 (63.4)	
Obesity	64 (44.1)	
Diabetes	32 (22.1)	
Previous lung disease	32 (22.1)	
Heart disease	31 (21.4)	
Chronic kidney disease	18 (12.4)	
Previous thrombosis	6 (4.10)	
Anticoagulation at ICU admission	95 (65.5)	LMWH:87, UFH:7, EDX:1, NA:50
Antiplatelet therapy at ICU admission	19 (13.1)	AAS:15; Clop:3; AAS+Clop:1
Clinical Scores	**Median**	**Variations**
SAPS II	43.5	11 - 105
SOFA	7.5	0 - 14
SIC	3	2 - 5
DIC	1	0 - 6

AAS: acetylsalicylic acid; DIC: disseminated intravascular coagulopathy; EDX: edoxaban; Clop: clopidogrel; ICU: Intensive care unit; IQR: Interquartile range; LMWH: Low molecular weight heparin; NA: Not available; SAPS II: simplified acute physiology score II; SIC: Sepsis-induced coagulopathy; SOFA: Sequential organ failure assessment; UFH: Unfractionated heparin.

Table 2 – Patient characteristics during Intensive Care Unit hospitalization

Parameter	n (%)	IQR
ICU length-of-stay (median) (days)	14	5 - 27
Hospital length-of-stay (median) (days)	26	14.25 - 43.75
Anticoagulation during ICU hospitalization:		
Day 5 (n = 125)	124 (99.2%)	LMWH:108; UFH:15; Biva:1
Day 30 (n = 35)	33 (94.3%)	LMWH:25; UFH:6; Biva:2
ICU Discharge (n = 145)	139 (95.99)	LMWH:131; UFH:9
Invasive mechanical ventilation	97 (66.9)	
ECMO support	26 (18.4)	
Thrombotic events	32 (22.1)	
Hemorrhagic events	22 (15.1)	
Mortality rate:		
Global	51 (35.0)	
At ICU	43 (29.7)	
At hospital (after ICU discharge)	8 (5.50)	

Biva: bivalirudin; ECMO: extracorporeal membrane oxygenation; ICU: Intensive Care Unit; IQR: interquartile range; LMWH: low molecular weight heparin; UFH: unfractionated heparin.

at admission *versus* at discharge are shown [full data is displayed in the supporting information (Appendix 2: https://www.actamedicaportuguesa.com/revista/index.php/amp/article/view/19475/15151)].

Lymphocyte and other blood counts, biochemical (LDH, PCT, iCa^{2+}, creatinine, GFR, AST, ALT, albumin, troponin), inflammatory (CRP, IL-6, ferritin), and coagulation (aPTT, fibrinogen, FVIII, antithrombin) parameters, as well as SOFA score, differed significantly ($p < 0.05$) between day of admission and of discharge (Appendix 1: https://www.actamedicaportuguesa.com/revista/index.php/amp/article/view/19475/15148). The same was seen concerning thromboelastometric findings of hypercoagulability (EXTEM-CFT; FIBTEM-A5, A10, MCF) and of hypofibrinolysis (EXTEM-LI60, ML; INTEM and HEPTEM-LI30, LI60, ML).

On the other hand, no statistically significant differences were seen for the other coagulation parameters (D-dimer, international normalized ratio (INR), vWF:Ag, and vWF:RCo), and for the SIC and DIC scores (Appendix 2: https://www.actamedicaportuguesa.com/revista/index.php/

Table 3 – Thromboelastometry parameters: hypercoagulability and hypofibrinolysis in survivors versus non-survivors at Intensive Care Unit (ICU) admission and day 30 of ICU hospitalization (percentage of patients)

Thromboelastometry	ICU Admission-DAY 0	Survivors (n = 94)	Non-survivors (n = 51)
Hypercoagulability	CFT-EXTEM < 50 sec.	37.2%	29.4%
	MCF-EXTEM > 68mm	61.7%	56.9%
	MCF-FIBTEM > 22mm	98.9%	96.0%
Hypofibrinolysis	LI60-EXTEM ≥ 97%	26.9%	42.9%
	ML-EXTEM < 5%	9.5%	23.5%
	LI30-INTEM ≥ 97%	95.7%	98.0%
	Day 30 at ICU	**Survivors** (n = 94)	**Non-survivors** (n = 51)
Hypercoagulability	CFT-EXTEM < 50 sec.	28.5%	28.5%
	MCF-EXTEM > 68mm	50.0%	50.0%
	MCF-FIBTEM > 22mm	57.1%	78.6%
Hypofibrinolysis	LI60-EXTEM ≥ 97%	50.0%	78.6%
	ML-EXTEM < 5%	37.5%	42.9%
	LI30-INTEM ≥ 97%	100%	100%

CFT: clot formation time; ICU: Intensive Care Unit; LI30: Lysis index at 30 minutes after CT; LI60: Lysis index at 60 minutes after CT; MCF: maximum clot firmness; ML: maximum lysis; sec: seconds; EXTEM/FIBTEM/INTEM: thromboelastometry assays.
Percentage of survivor versus non-survivor patients showing the described thromboelastometry parameters at Intensive care unit (ICU) admission and on day 30 of ICU hospitalization.

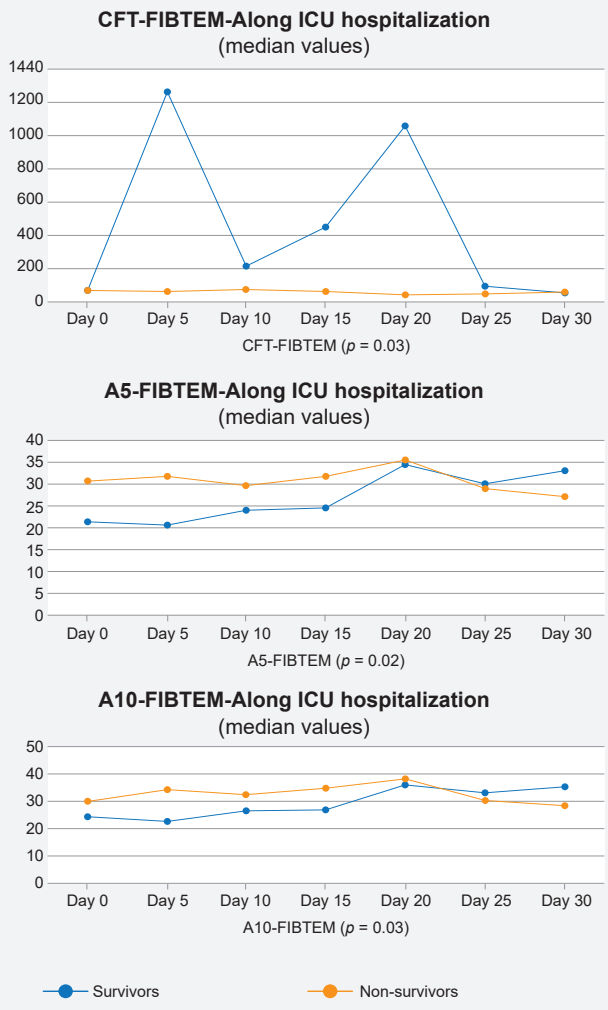

Figure 1 – Hypercoagulability along 30 days of Intensive Care Unit hospitalization: comparison between survivors (n = 94) and non-survivors (n = 51) (median values).
A5: amplitude at 5 minutes after CT; A10: amplitude at 10 minutes after CT; CT: clotting time; CFT: clot formation time; ICU: Intensive Care Unit. FIBTEM thromboelastometry assay.
[*p*-value from Appendix 1 (Appendix 1: https://www.actamedicaportuguesa.com/revista/index.php/amp/article/view/19475/15148)].

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During ICU hospitalization (Appendix 1: https://www.actamedicaportuguesa.com/revista/index.php/amp/article/view/19475/15148), a slightly higher number of lymphocytes from day ten (D10) was observed, but median values over $1x10^6$/L were only seen at D30 and at ICU discharge. A slight decrease in the level of inflammatory parameters (CRP, ferritin) was observed, although elevated levels were still observed at D30 and ICU discharge. However, normal median values of IL-6 were seen consistently from D15.

During the ICU stay, hypercoagulability was expressed through consistent persistently high levels of FVIII, vWF:Ag, vWF:RCo, and by thromboelastometry parameters (FIBTEM: A5, A10, MCF). Hypofibrinolysis expressed by thromboelastometry parameters (mainly by median values of INTEM/HEPTEM - LI30, LI60, ML), was also observed throughout ICU hospitalization, mostly until D30 (Appendix 1: https://www.actamedicaportuguesa.com/revista/index.php/amp/article/view/19475/15148). For all the other evaluated parameters, there were no significant differences between the different time points (Appendix 2: https://www.actamedicaportuguesa.com/revista/index.php/amp/article/view/19475/15151).

Comparing data from survivors and non-survivors, at ICU admission and on D30 of ICU stay (Table 3), patients presented with hypercoagulability and hypofibrinolysis, as defined elsewhere,[28,29] with greater incidence and severity in non-survivors. Major and significant differences between survivors and non-survivors were observed regarding the presence and severity of hypofibrinolyis, being more pronounced in non-survivors (Table 3, Figs. 1 and 2).

Higher hypercoagulability (FIBTEM: CFT, A5 and A10) (Fig. 1) and greater hypofibrinolysis in all assays (EXTEM/HEPTEM: LI60 and ML; INTEM: LI30, LI60 and ML) (Fig. 2) were observed in non-survivors compared with survivors along the 30 days of ICU hospitalization.

Most patients received prophylactic and intermediate doses of LMWH. No differences in the hypercoagulability and hypofibrinolysis profiles were observed in thromboelastometry between patients receiving prophylactic and intermediate heparin doses.

DISCUSSION

HemoCoV is a real world, prospective and non-interventional study that evaluated COVID-19-associated coagulopathy (CAC) and its implications in the clinical course of SARS-CoV-2 infection along 30 days of ICU hospitalization. One hundred and forty-five consecutive patients with COVID-19 moderate to severe acute respiratory failure were assessed for extensive evaluation of coagulation parameters, using conventional tests and thromboelastometry. Despite anticoagulation treatment, thromboelastometry still revealed significant coagulation abnormalities expressed by hypercoagulability and hypofibrinolysis, since ICU admission (initial phases of disease) and persisting throughout the clinical course. Both changes were more pronounced in patients with higher disease severity and in non-survivors. More prevalent and severe hypofibrinolysis was observed in non-survivors. This may reflect a significant role of hypofibrinolysis in the pathophysiology of severe COVID-19.

Similar results have been previously described,[33-36] suggesting that thromboelastometry parameters may be useful to distinguish coagulation patterns between patients with non-severe and severe COVID-19.[35] Some authors additionally suggest that this phenotypic characterization may be useful for personalized therapeutic intervention.[35] None of our patients had CT-EXTEM under 45 seconds, often

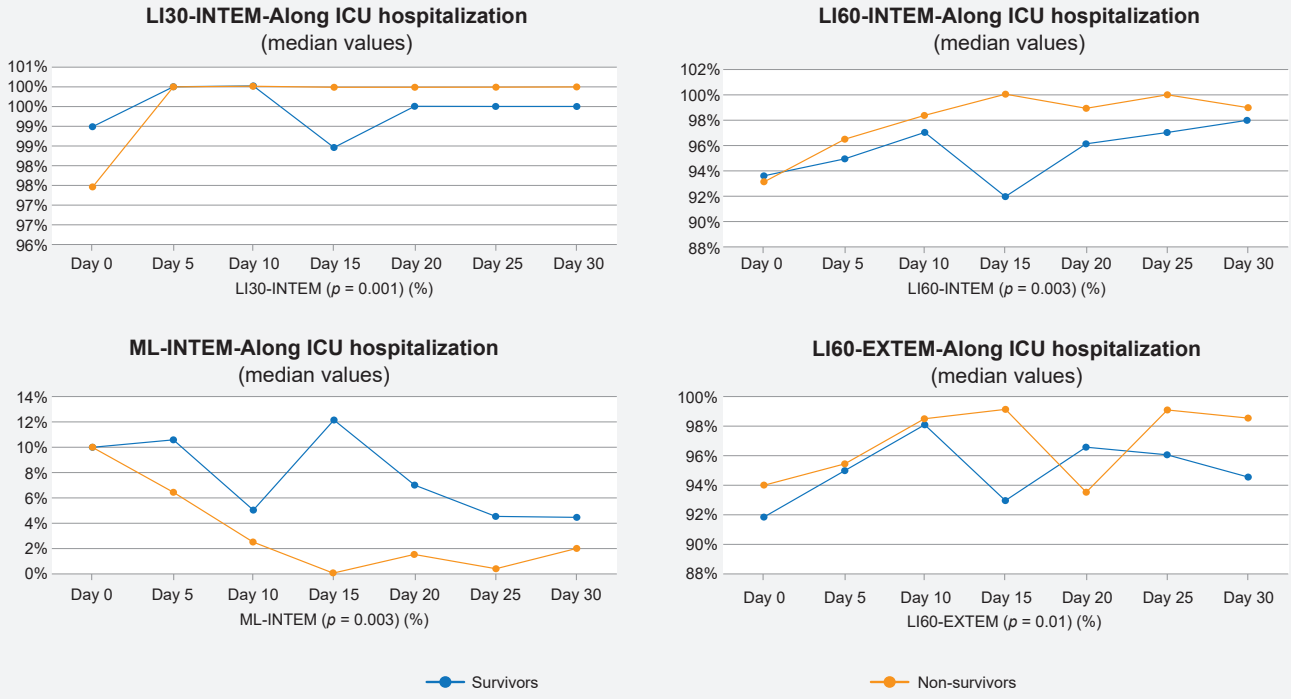

Figure 2 – Hypofibrinolysis along 30 days of Intensive Care Unit hospitalization: comparison between survivors (n = 94) and non-survivors (n = 51) (median values).
CT: clotting time; ICU: Intensive Care Unit; MCF: maximum clot firmness; LI30: Lysis index at 30 minutes after CT; LI60: Lysis index at 60 minutes after CT; ML: maximum lysis; Maximum lysis (ML): is the reduction of clot firmness after the MCF. INTEM/EXTEM thromboelastometry assays.
[*p*-value from Appendix 1 (Appendix 1: https://www.actamedicaportuguesa.com/revista/index.php/amp/article/view/19475/15148)].

linked to hypercoagulability,[28] as previously described in other studies.[35] The higher burden of fibrinogen and other coagulation factors (FVIII, vWF) along the clinical course of COVID-19, was observed in our study also by the elevated levels of CT-FIBTEM and no significant differences between ICU admission and discharge. To stress the clinical importance of hypercoagulability, some authors[35,37] propose a cut-off for MCF-FIBTEM over 27mm to differentiate between severe and non-severe forms of COVID-19 disease, with high accuracy.[35] In our patients the median values of MCF-FIBTEM were above the normal range from ICU admission to discharge, although showing a significant difference (p = 0.02). Concerning fibrinolysis, there have been suggestions of a functional shutdown in patients with severe COVID-19.[38-40] This description matches our findings, which revealed decreased fibrinolysis, suggesting a prothrombotic condition and thus compromising vascular permeability. This finding is not always reproducible,[35] which highlights the complex interactions of the coagulation system.

D-dimer levels in our population did not differ significantly between time point analyses, despite previous suggestions of it being a reliable predictor of disease severity or a prognostic marker for in-hospital mortality.[41] Conversely, as others described,[42] fibrinogen levels were significantly different from ICU admission to discharge (p < 0.001), and high prevalence of increased fibrinogen levels (> 7 g/L) at admission[43] were observed. Hence, a careful assessment of fibrinogen is required for stratifying CAC, which may have been overlooked and may need to be revisited.[42]

As previously described in the recent literature,[44] severe lymphopenia was identified in our study with a statistically significant difference in lymphocyte count between ICU admission and discharge (p < 0.001), reflecting the severity of this viral disease.

To the best of our knowledge, our study is one of the first where different coagulation tests (standard coagulation and viscoelastic tests) as well as biochemical and clinical parameters were prospectively and extensively analyzed in COVID-19 patients during 30 days of ICU hospitalization. This allowed us to analyze CAC with a holistic view, identifying coagulation abnormalities that may have a clinical impact on future evaluation and treatment of these patients. One of the strengths of our study is the longitudinal temporal characterization of coagulation abnormalities. Almost all coagulation tests evaluated differed from the initial to the final stages of the disease. The changes documented in our

study strongly suggest that pathophysiological mechanisms involved in COVID-19 may be related to a hyperthrombotic profile along with hypofibrinolysis. This could be extremely useful in clinical practice, namely for defining distinctive therapeutic interventions, such as anticoagulation implementation and dosage, and for stratification of the patient's individual risk in subsequent studies. Moreover, and by being a single center study, it allowed us to ensure similar established interventions and homogeneous therapeutic and intensive care interventions.

Our study has several limitations. Firstly, it is a single-center, open-label, and non-randomized study, with a specific cohort that might not be representative of other populations. As genetic factors are known to affect the coagulation system, our findings should be reproduced and evaluated in other settings and other genetic backgrounds. Additionally, it should be recognized that this study took place during the SARS-CoV-2 delta variant pandemic wave, and hence there is no irrevocable evidence that similar results would occur with other coronavirus variants.

CONCLUSION

Our study found that hypercoagulability and hypofibrinolysis, as assessed by rotational thromboelastometry, are present from ICU admission and persist throughout the clinical course of severe COVID-19 patients, despite receiving anticoagulation treatment. This profile was more pronounced in patients with higher disease severity. Hypofibrinolysis was more prevalent and more severe in non-survivors, which may reflect its significant role in the pathophysiology of severe COVID-19. Better characterization of COVID-19-associated coagulopathy might help identify severe COVID-19 disease and poor outcomes.

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AWARDS AND PREVIOUS PRESENTATIONS

This work was presented at the 35th European Society of Intensive Care Medicine (ESICM) Annual Congress, on 22nd – 26th October 2022, in Paris, France; and at the XII National Congress of Association Portuguese of Immunehemotherapy (Transfusion Medicine) (APIH), on 14th – 15th October 2022, in Figueira da Foz, Portugal.

AUTHOR CONTRIBUTIONS

AR: Concept and design of the study, data collection, analysis and interpretation of data, critical writing, revision and approval of the final version.

TDD, GNJ: Analysis and interpretation of data, critical writing, revision and approval of the final version.

AG, ARR, CJC: Data collection, analysis and interpretation of data, revision and approval of the final version.

CLP: Data collection, revision and approval of the final version.

DC: Data collection, analysis and interpretation of data, revision and approval of the final version.

AB: Revision and approval of the final version.

JMR: Analysis and interpretation of data, revision and approval of the final version.

PROTECTION OF HUMANS AND ANIMALS

The authors declare that the procedures were followed according to the regulations established by the Clinical Research and Ethics Committee and to the Helsinki Declaration of the World Medical Association updated in 2013.

DATA CONFIDENTIALITY

The authors declare having followed the protocols in use at their working center regarding patients' data publication.

COMPETING INTERESTS

The authors have declared that no competing interests exist.

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ACTA MÉDICA PORTUGUESA
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Acute Hepatitis of Unknown Origin in Children: Two Cases in a Portuguese Hospital

Hepatite Aguda de Origem Desconhecida em Crianças: Dois Casos num Hospital Português

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ABSTRACT

Several cases of paediatric acute hepatitis of an unknown aetiology have been described in these last few months and in several countries worldwide. We present two patients, a 7-month-old girl and an 8-year-old boy, with gastrointestinal symptoms and lethargy, associated with elevation of transaminase levels. Serologies for hepatitis A-E virus and PCR test to SARS-CoV-2 were all negative. In the first case, an adenovirus serotype C could be isolated in a respiratory sample as well as cytomegalovirus (CMV) in the blood (100 copies/mL). In both children, there was a progressive decrease in the hepatic markers and symptomatic resolution, compatible with a good prognosis, also seen globally in most cases. To date, infection remains the most plausible cause to consider, especially when it is presumed to be linked to adenovirus. Other potential agents and causes are still being evaluated, thus emphasizing the importance of continuous epidemiological surveillance, notification, and detailed study of all hepatitis cases.
Keywords: Acute Disease; Child; Disease Outbreaks; Hepatitis; Portugal

RESUMO

Casos de hepatite aguda de origem desconhecida têm sido descritos em idade pediátrica nos últimos meses e em vários países por todo o mundo. Apresentamos dois casos, uma lactente de sete meses e uma criança de oito anos, com sintomas gastrointestinais e prostração, associados a elevação das transaminases. As serologias para vírus da hepatite A-E e a pesquisa por PCR de SARS-CoV-2 foram negativas. Na lactente isolou-se adenovírus serotipo C nas secreções respiratórias e citomegalovírus (CMV) no sangue (100 cópias/mL). Em ambos houve uma descida progressiva dos marcadores hepáticos e resolução sintomática, compatível com o bom prognóstico que se tem verificado globalmente na maioria dos casos. A etiologia infeciosa é a hipótese mais plausível, sobretudo a infeção por adenovírus, mas outras causas têm também sido propostas, realçando a importância da vigilância epidemiológica, notificação e estudo detalhado de todos os casos de hepatite.
Palavras-chave: Criança; Doença Aguda; Hepatite; Portugal; Surtos de Doenças

INTRODUCTION

By the end of March 2022, the Scottish National Health Service raised concerns about a large number of children under 16 years old presenting with symptoms compatible with acute hepatitis (AH) not caused by the usual viral suspects.[1] Several hypotheses are still being considered to justify this strange rise in paediatric AH incidence,[2,3] with the infectious cause being quite appealing to the scientific community as an adenovirus (41F) has been identified in a large proportion of patients.[4] Other studies also suggested a potential role of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), or even other toxic agents, but no research has yet brought complete light on the matter.[4-6] The current proposed case definitions also require high transaminase levels (aspartate aminotransferase (AST) or alanine aminotransferase (ALT) above 500 UI/L),[2-4] which may suggest that milder cases are not being reported, probably underestimating the real number of affected children.

The most common clinical presentation consists of jaundice preceded or accompanied by vomiting.[4,7] However, other gastrointestinal (GI) symptoms have also been commonly reported, such as diarrhoea, pale stools, nausea and abdominal pain.[4,7] Severe lethargy is also a frequent complaint[4] and, in some cases, respiratory symptoms are also reported.[4,7]

As of 26th May 2022, the World Health Organisation had reported 650 probable cases of AH in 33 countries worldwide, 34% of these located in the United Kingdom (UK).[2] In the UK, most cases were children under five years of age (75.4%), around 60% were positive for an adenovirus (110 of the 181 tested),[2] 180 children required hospitalisation and 11 of those cases required liver transplantation.[4] There were no deaths recorded over one month of follow-up.[4]

Until the 3rd June 2022, in Portugal, 15 children remained under investigation and surveillance.[8]

We present two cases, whose initial symptoms report back to the 31st March and the 2nd April, 2022, respectively, making them possibly some of the first cases notified in our country.

CLINICAL CASES

A previously healthy 7-month-old girl presented to our paediatric emergency department (PED) on the 6th April

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2022, with a six-day history of diarrhoea and vomiting, and also with persistent fever and lethargy for the previous four days. There were no changes in the physical examination apart from mild dehydration. She was anicteric and there was no reported coluria or faecal acolia. There was also no recent significant epidemiological context and she had had coronavirus disease 2019 (COVID-19) in January 2022. Serial blood workup over a three-week period showed elevated ALT (maximum level 1386 U/L), AST (maximum level 1059 U/L), and gamma glutamyl transpeptidase (gamma-GT) (maximum level 168 U/L). Prothrombin time (PT), activated partial thromboplastin time (aPTT) and total bilirubin levels were normal (Table 1). The respiratory viral panel was positive for adenovirus and coronavirus HKU1. Serologies were negative for hepatitis A-E virus and Epstein-Barr virus (EBV), only showing a positive IgG for cytomegalovirus (CMV), with negative IgM. Complementary investigation performed by Instituto Ricardo Jorge (INSA) on blood, serum and faecal samples revealed: positive C serotype adenovirus desoxyribonucleic acid (DNA) detected by polymerase chain reaction (PCR) method on a faeces sample (but negative in the blood), with positive IgG and negative IgM on serology; positive DNA PCR for CMV in the blood (100 copies/mL); negative DNA PCR for EBV in the blood; and positive IgG-anti-nucleoprotein and anti-S protein for SARS-CoV-2.

Around the same time, a previously healthy 8-year-old boy was also brought to our PED with complaints of anorexia and severe lethargy with seven days duration, accompanied by diffuse abdominal pain, vomiting, cough and rhinorrhoea, which had started a few days earlier. There was no fever, diarrhoea or reported changes in faeces or urine. According to his parents, there had been no relevant epidemiological link, including previous COVID-19, and the boy had not been vaccinated for SARS-CoV-2. His blood workup was relevant for elevation of transaminases (AST max. of 1081 U/L, ALT max. of 1999 U/L), and elevated lactate dehydrogenase (LDH) of 651 U/L. There were no other relevant changes, including bilirubin, albumin and coagulation studies (Table 2). The virus panel testing was negative for hepatitis A-E virus, EBV, CMV, SARS-CoV-2 and additional investigation performed at INSA revealed inconclusive IgG with negative IgM, and negative DNA PCR for adenovirus; negative DNA PCR for CMV and EBV; negative RNA PCR for enterovirus; negative IgG anti-nucleoprotein but positive IgG anti-S protein for SARS-CoV-2.

Over a two-month follow-up, both children normalized their transaminase levels and have fully recovered from their symptoms.

DISCUSSION

So far, an infectious agent remains the most plausible cause as it is shown in one of the presented cases.[4-7] Both children had GI symptoms at presentation and both had frank elevation on hepatic markers, although none presented with jaundice or pale stools, which are a common feature

Table 1 – Laboratorial evaluation of patient 1 (7-month-old girl)

	2022/04/06	2022/04/10	2022/04/13	2022/04/27	Reference values*
Hb (g/dL)	10.9	11.4	11.2	11.3	10.5 – 14.0
Leuc (cel/uL)	9900	7600	10100	8700	6000 – 14 000
Plat (cel/uL)	**173 000**	**84 000&**	321 000	334 000	150 000 – 400 000
PT (sec)		12.43	12.73	11.2	10.3 – 12.8
INR		1.16	1.19	1.06	
aPTT (sec)		23.48	**34.46**	27.70	21.6 – 28.7
pCr (mg/dL)	**4.38**	0.36	0.17		0.08 – 1.12
AST (U/L)	**758**	**1059**	**782**	**219#**	22 – 63
ALT (U/L)	**838**	**1386**	**677**	**259#**	8 – 32
GGT (U/L)	25	**168**	**107**	29	5 – 32
LDH (U/L)		**970**	**715**	385	150 – 580
Total bilirubin (mg/dL)		0.4		0.38	< 1.00
Direct bilirubin (mg/dL)		**0.21**		0.12	0.00 – 0.20
Indirect bilirubin (mg/dL)		0.19		0.26	0.20 – 0.80

ALT: alanine aminotransferase; aPTT: activated partial thromboplastin time; AST: aspartate aminotransferase; GGT: gamma-glutamyl transferase; Hb: haemoglobin; INR: international normalized ratio; LDH: lactate dehydrogenase; Leuc.: leucocyte count; Plat: platelet count; PT: prothrombin time; sec: seconds

* Normal range reference values for age stated on Nelson's Textbook of Pediatrics (Chapter 748, 21st Edition, published in 2020) were considered. Reference values not present in this chapter were substituted for those considered by the laboratory at our institution.

By May 24th 2022, AST was of 47 U/L and ALT was of 37 U/L (values obtained in outpatient work-up).

& No pseudo-thrombocytopenia was detected by our laboratory and a double confirmatory essay was performed in the same sample; the patient was not submitted to any platelet transfusion.

Table 2 – Laboratorial evaluation of patient 2 (8-year-old boy)

	2022/04/09	2022/04/10	2022/04/11	Reference values*
Hb (g/dL)	13.8	12.4		11.5 – 14.5
Leuc (cel/uL)	11500	5500		4000 – 12 000
Plat (cel/uL)	22300	215 000		150 000 – 400 000
PT (sec)	12.38	**12.97**		10.3 – 12.8
INR	1.16	1.21		
aPTT (sec)	23.18	25.80		23.0 – 31.9
pCr (mg/dL)	0.22	0.14	0.13	0.06 – 0.79
AST (U/L)	**1081**	**651**	**391#**	15 – 50
ALT (U/L)	**1999**	**1656**	**1408#**	5 – 45
GGT (U/L)	26	29	**42**	5 – 32
LDH (U/L)	**651**	426		150 – 500
Total bilirubin (mg/dL)	0.9	**1.0**		< 1.00
Direct bilirubin (mg/dL)	**0.29**	**0.39**		0.0 – 0.20
Indirect bilirubin (mg/dL)	0.61	0.70		0.20 – 0.80

ALT: alanine aminotransferase; aPTT: activated partial thromboplastin time; AST: aspartate aminotransferase; GGT: gamma-glutamyl transferase; Hb: haemoglobin; INR: international normalized ratio; LDH: lactate dehydrogenase; Leuc.: leucocyte count; Plat: platelet count; PT: prothrombin time; sec. seconds

* Normal range reference values for age stated on Nelson's Textbook of Pediatrics (Chapter 748, 21st Edition, published in 2020) were considered. Reference values not present in this chapter were substituted for those considered by the laboratory at our institution.

By May 17th 2022, AST was of 26 U/L and ALT was of 22 U/L (values obtained in outpatient work-up).

in other case series.[2-4,7] Our first patient had fever, a sign present in about 30% of the previously described cases,[4,7] and the second patient also had respiratory symptoms, as seen in around 19% of those reported, so far, in the literature.[4,7]

As in the majority of cases in the UK,[4,5] an adenovirus was also detected in our first case (in the respiratory panel and stools), although the revealed serotype in our case was not the one being currently considered as the most likely culprit by the international scientific community (the 41F serotype, not previously known to cause severe AH).[4,9] Besides, in the UK, this adenovirus is being more easily detected in the blood or serum than in stool or respiratory samples,[4] contrary to what happened in our case, where no adenoviral DNA could be detected in blood PCR.

Although the adenovirus hypothesis is a substantial focus of investigation, there are some questions about its true role, since it is a frequent infection in children and can be only an incidental finding. Moreover, between November 2021 and April 2022, England's health surveillance system registered more positive tests for adenoviral infection in 1- to 4-year-old-children, than in comparable periods in the previous five years, which could suggest a post-pandemic peak, unrelated to the surge of new AH cases.[4]

Additionally, in our first case, CMV was detected by PCR method on the blood which could have had justified the whole clinical picture. But since there were negative IgM antibodies and the detected viral load detected by the PCR method was low, at around 100 copies/mL, we consider this virus an unlikely aetiology for the AH. The detection of low levels of CMV by the PCR method could be explained by a reactivation of previous infections, in a context of a probable immunocompromised state, similar to the reactivation of EBV seen in patients who go through stem cell transplantation.[5] There are reports of positive EBV by PCR testing with negative EBV IgM antibodies in the context of some these unexplained hepatitis cases, and even other agents such as CMV have also been identified in some cases, although in lower frequency.[5-7,9]

Another possible theory is that all these newly identified AH cases can be due to acute COVID-19 (with perhaps a new variant and incidental isolation of other viruses) or that we could be looking at yet another form of a post infectious SARS-CoV-2 syndrome.[4,6,7,9]

Weeks after the SARS-CoV-2 delta variant outbreak, there were reports in India of 37 children with mild hepatitis with preserved synthetic liver function and no jaundice.[7,10] Although these seem to be less severe than the newly reported AH cases, it cannot be ruled out that they can still represent a more severe end of spectrum of the same condition.[7] However, this seems to be unlikely, since most children with AH in the UK and other countries have, so far, tested negative for SARS-CoV-2 acute infection on admission.[4,6,7,9] Nevertheless, it is logical that most of these children had had a previous COVID-19 infection and serological testing has been supportive of this fact.[9]

Another prominent hypothesis to consider is the possibility of a shared role between SARS-CoV-2 and other

viruses (such as adenovirus), in the pathophysiology of these unexplained hepatitis cases.[4] Investigations on how SARS-CoV-2 could alter the immune response to infection, allowing for a more severe adenoviral infection are ongoing.[4,6,7,9] There are current theories that infection with SARS-CoV-2 can lead to the establishment of a viral reservoir persisting in the GI tract, and progressively releasing viral proteins across the epithelium, leading to constant non-specific T-cell activation.[6] This unceasing activation is most likely due to a superantigen motif in the spike protein that resembles staphylococcal enterotoxin B.[6] It is known that, in mice models, infection with adenovirus makes them more susceptible to subsequent staphylococcal enterotoxin B-mediated toxic shock, which leads to liver failure and death, so extrapolating the data to an *in vivo* scenario allows us to speculate that a child with previous SARS-CoV-2 infection and possible persistence of viral reservoir, infected with an adenovirus with GI-trophism (such as F41), could suffer from an immune deregulatory state that could culminate in severe AH and eventual liver failure.[6]

Basal immune dysregulation or suppression is thought to contribute in some part to explain these cases, but evidence is still lacking.[4-7,9] Physical isolation during the pandemic gave rise to a lack of exposure to common pathogens, resulting in an immunodeficiency state-like, with higher susceptibility to other viruses after relaxation of the pandemic restrictions, which therefore allowed for rarer outcomes of common infections to be detected.[4,7]

Concerning SARS-CoV-2, in our first case, the positive IgG anti-nucleoprotein and anti-S protein for SARS-CoV-2, indicates a previous infection corroborated by the clinical history. In the second case, negative IgG anti-nucleoprotein but positive IgG anti-S protein for SARS-CoV-2 remains a mystery as the child's mother denies any previous (at least symptomatic with laboratory evidence) infection and the boy had not been vaccinated, but we speculate it may also suggest previous infection not diagnosed in the acute state by nasopharyngeal swab PCR or other similar method. As is internationally reported, our two cases have not been vaccinated which makes a post-vaccine reaction an unlikely scenario.[4-7]

Both of our cases had a favourable outcome, like most literature reports, and neither child had synthetic liver failure, with coagulation issues, or need of transplant.

As the origin of these severe AH cases remains overall still a mystery for the scientific and medical community, it is important to notify them according to regional and national protocols.

These cases should make us wonder about these children's basal immune status, the role SARS-CoV-2 may play in a possible upregulation and explore other potential underlying causes, besides considering adenovirus upfront as the most likely suspect.

The prognosis appears to be good in most cases, even though there is a non negligible risk of acute hepatic failure and need of transplant. As such, prompt identification and close follow-up are required.

AUTHOR CONTRIBUTIONS

RCC: Draft of the article, data acquisition and analysis.

CSE: Critical review of the manuscript, contribution to data analysis and interpretation.

PF, LV: Design of the article, critical review of the manuscript, final approval of the version to be published.

PROTECTION OF HUMANS AND ANIMALS

The authors declare that the procedures were followed according to the regulations established by the Clinical Research and Ethics Committee and to the Helsinki Declaration of the World Medical Association updated in 2013.

DATA CONFIDENTIALITY

The authors declare having followed the protocols in use at their working center regarding patients' data publication.

PATIENT CONSENT

Obtained.

COMPETING INTERESTS

The authors have declared that no competing interests exist.

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ACTA MÉDICA PORTUGUESA
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Inflamação na Cicatriz BCG após Primeira Dose de Vacina mRNA Anti-SARS-CoV-2

Inflammation of BCG Inoculation Site Scar after the First Dose of an Anti-SARS-CoV-2 mRNA Vaccine

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RESUMO

Reações inflamatórias no local da cicatriz da vacina Bacillus Calmette-Guérin (BCG) foram previamente descritas em relação a infeções virais. A inflamação da cicatriz da BCG em relação à administração de vacinas foi reportada com a vacina da gripe, e, mais recentemente, após a segunda dose das vacinas mRNA anti-SARS-CoV-2 disponíveis (mRNA-1273 e BNT162b2), em dois profissionais de saúde. Aqui apresentamos o caso de uma jovem de 27 anos, sem história pregressa relevante, incluindo infeção por SARS-CoV-2, com inflamação da cicatriz da BCG oito dias após a primeira administração de vacina mRNA anti-SARS-CoV-2. A farmacovigilância e a notificação de reações adversas devem ser incentivadas, de forma a não criar hesitação nesse processo.
Palavras-chave: Cicatriz; Efeito Colateral e Reação Adversa Relacionados a Medicamentos; mRNA SARS-CoV-2; Vacina BCG; Vacinas contra COVID-19; Vacinas de mRNA

ABSTRACT

Inflammatory reactions in the Bacillus Calmette-Guérin (BCG) inoculation scar site have been previously described, in association with viral infections. The inflammation of the scar in association with other vaccines has been described with the flu vaccine and, more recently, after the second dose of mRNA anti-SARS-CoV-2 vaccines (mRNA-1273 e BNT162b2), in two healthcare workers. We present the case of a 27-year-old female, without a relevant past medical history, including no previous SARS-CoV-2 infection, and with inflammation of the BCG scar eight days after the first dose of the mRNA anti-SARS-CoV-2 vaccine. Pharmacovigilance and the notification of adverse events should be encouraged, as a way of warding off hesitation in this process.
Keywords: BCG Vaccine; Cicatrix; COVID-19 Vaccines; Drug-Related Side Effects and Adverse Reactions; mRNA Vaccines; SARS-CoV-2

INTRODUÇÃO

A inflamação local da cicatriz da Bacillus Calmette-Guérin (BCG) tem sido reportada maioritariamente em crianças com doença de Kawasaki (DK),[1] sarampo e herpesvirus 6,[2] sendo caracterizadas por eritema, indução, formação de crosta ou até ulceração no local da cicatriz.

A inflamação da cicatriz da BCG após imunização foi reportada após vacinação contra a gripe,[3] e, mais recentemente, em dois casos após a administração da segunda dose das duas vacinas mRNA anti-SARS-CoV-2 disponíveis (BNT162b2 e mRNA-1273).[2] Aqui descrevemos um dos primeiros casos reportados de inflamação da cicatriz da BCG, numa jovem de 27 anos saudável, sem história pregressa relevante, oito dias após a administração da primeira dose da vacina mRNA anti-SARS-CoV-2.

CASO CLÍNICO

Uma jovem de 27 anos do sexo feminino, fumadora e sem uso de medicação habitual, sem história prévia de infeção por SARS-CoV-2, vacinada com a BCG ao nascimento no seu braço esquerdo, recebeu a primeira dose da vacina mRNA-1273 COVID-19 (Moderna®), também no braço esquerdo, sem sintomas nem intercorrências imediatas, não reportando alterações adicionais nos dias seguintes, nomeadamente, rubor, calor, alteração de pigmentação ou exsudado.

Oito dias após a administração da vacina mRNA anti-SARS-CoV-2, a jovem desenvolveu uma reação de forma redonda e bordos mal definidos com cerca de 2 cm de diâmetro no local da cicatriz da BCG (Fig.1), caracterizada por eritema, indução e dor, e distanciada cerca de 4 cm do local de inoculação da vacina anti-SARS-CoV-2 (Fig. 2). A doente não reportou febre, mialgias nem outros sintomas. Não foram detetadas alterações no exame objetivo, nomeadamente adenopatias.

Os sintomas persistiram por três dias, sendo que a doente optou por não ser medicada. Passados 11 dias desde o início dos sintomas, foi feito estudo analítico complementar (Tabela 1), revelando resultado positivo para IgG anti-SARS-CoV-2, estando os restantes valores dentro da normalidade, incluindo linfócitos e serologias víricas. O incidente foi submetido como possível reação adversa a medicamentos no Sistema Nacional de Farmacovigilância.

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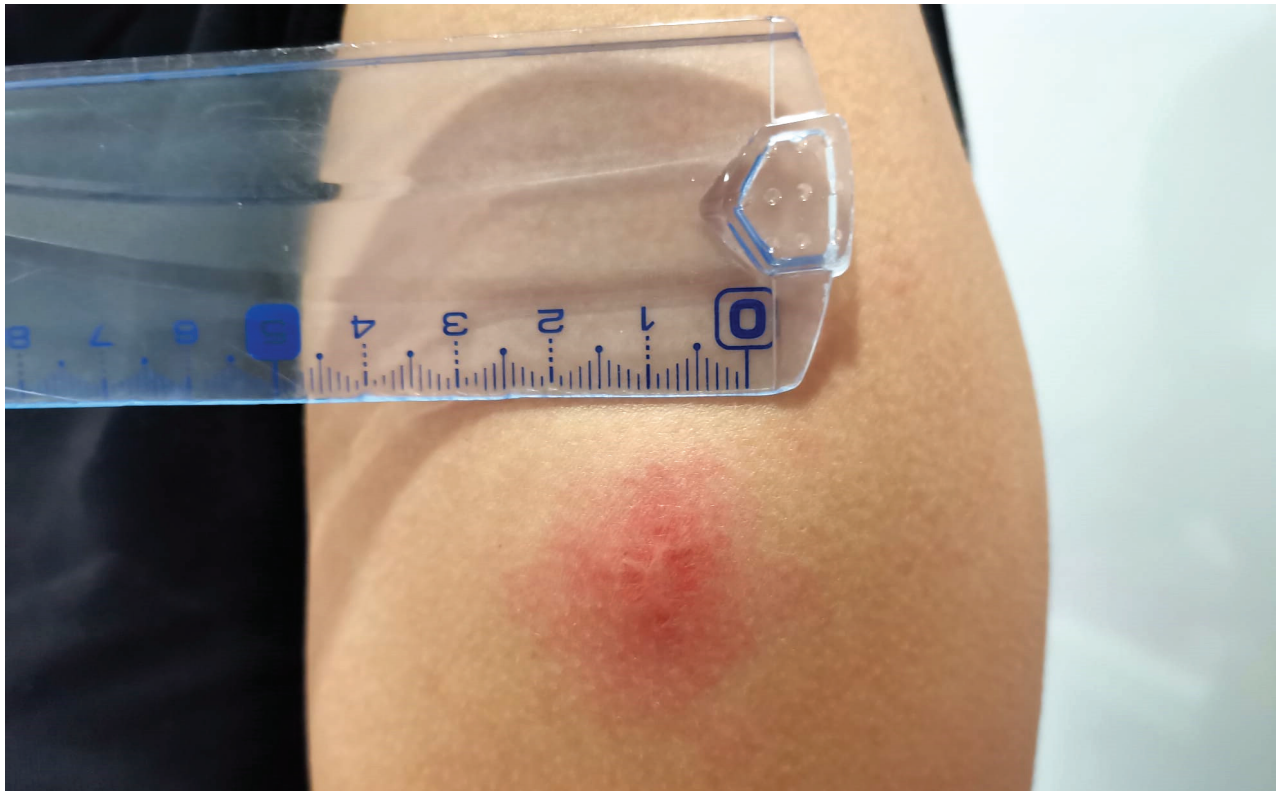

Figura 1 – Inflamação e indução no local da BCG, sendo visível a superfície elevada e irregular

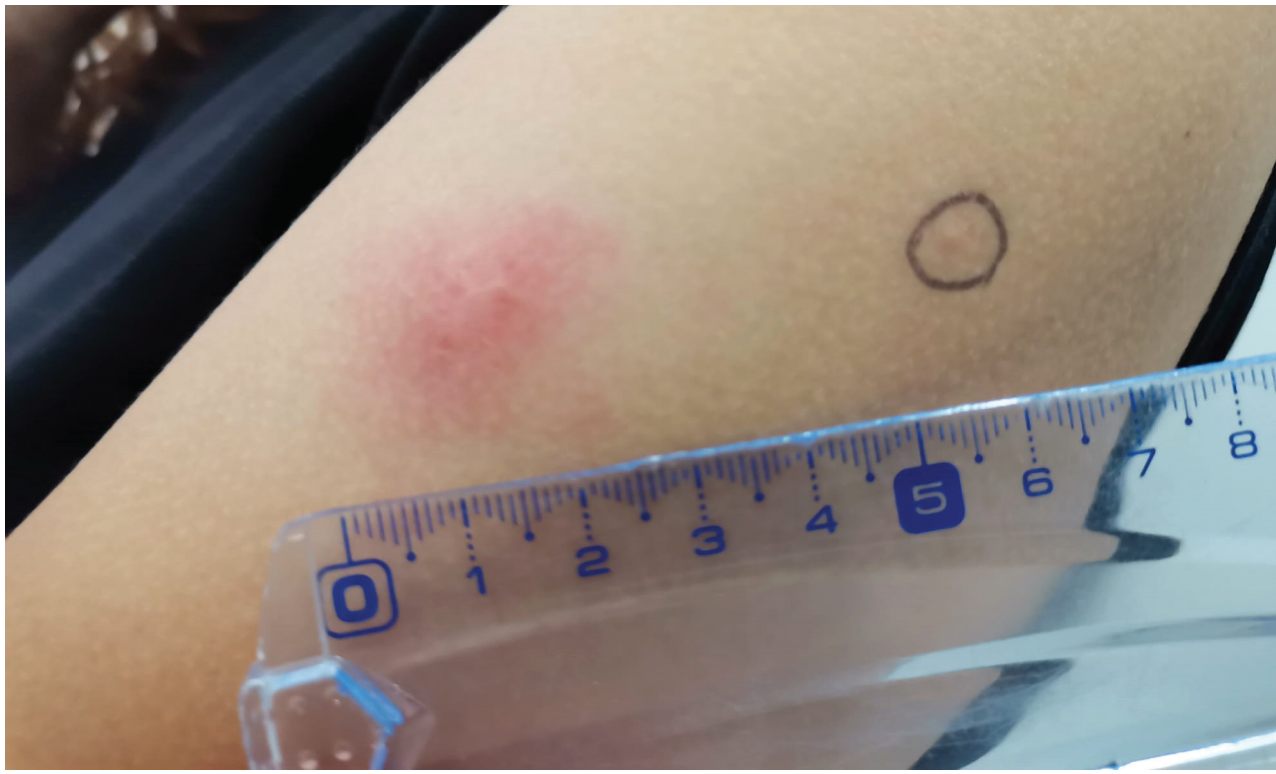

Figura 2 – Relação espacial entre a inoculação da vacina anti-SARS-CoV-2 e a inflamação do local da BCG

Tabela 1 – Resultados laboratoriais

Resultados laboratoriais			
Leucócitos	9,16x10³/uL	Ac. Anti SARS-CoV-2 IgG	246,3 AU/mL
Neutrófilos	45,7%	IgG Herpes 1/2	Negativo
Eosinófilos	5,7%	IgG EBV	Positivo
Basófilos	0,5%	IgM EBV	Negativo
Linfócitos	42,0%	AgHBs	Negativo
Monócitos	6,1%	AcHBs	Negativo
Hemoglobina	15,1 g/dL	Ac HCV	Negativo
Plaquetas	223x10³/uL	Ag+Ac HIV I/II	Negativo
Velocidade de sedimentação	1mm/h		

DISCUSSÃO

A vacina BCG é composta por uma estirpe atenuada de *Mycobacterium bovis,* interagindo com componentes do sistema imunitário no local da inoculação, gerando uma resposta imune intensa e um granuloma característico no qual permanecem células responsáveis pela hipersensibilidade tardia (tipo IV).[2]

O significado clínico deste fenómeno relativamente à resposta imunológica à vacinação para SARS-CoV-2 é desconhecido.

Em países como Portugal, onde a cobertura de BCG é praticamente universal, reações como esta são de particular interesse, pois poderão causar preocupação e gerar ansiedade e hesitação nos doentes ainda não vacinados, ou que receberão dose de reforço.

A inflamação da cicatriz tem vindo a ser descrita em relação com processos imunomediados como a DK.[1,4] O mecanismo subjacente ainda não é bem compreendido, mas poderá ser explicado pelas reações cruzadas entre as proteínas de choque térmico (*heat shock protein* - HSP) homólogas (micobacterianas e humanas), podendo desempenhar um papel no dano tecidual característico da DK. De forma semelhante aos casos descritos, a libertação de HSP pela vacinação poderá ter estimulado a resposta imune na cicatriz da BCG. Foi sugerido que a libertação de HSP após inoculação com a vacina contra influenza poderá estimular uma resposta imune semelhante, resultando na resposta inflamatória na cicatriz da BCG.[5] Os mecanismos para estes achados permanecem por esclarecer, e este fenómeno manteve a sua raridade, apesar da elevada percentagem (87%)[6] de população vacinada em Portugal.

Um artigo recente veio a revelar uma associação entre a vacinação com BCG e uma resposta de citocinas ampliada após exposição à gripe, e, de forma menos intensa, ao SARS-CoV-2. Além disso, em doentes com COVID-19, a resposta sorológica após a infeção foi significativamente mais forte no grupo vacinado com BCG.[7] No caso aqui exposto assumimos o resultado positivo para IgG anti-SARS-CoV2 como a reação expectável à inoculação, conferindo o estado de vacinado.

CONCLUSÃO

Na data de submissão deste manuscrito, este era o primeiro caso reportado sobre esta reação à primeira dose da vacina mRNA anti-SARS-CoV-2, tendo existido entretanto publicação de outro caso semelhante.[8] Foram previamente publicados dois casos, relativos à segunda dose da vacina mRNA anti-SARS-CoV-2. Todos os profissionais de saúde devem ter em conta todas as reações adversas reportadas, de forma a notificar e abordar este fenómeno e a minimizar queixas e preocupações por parte dos pacientes, que de outra forma poderão ter um impacto negativo na vacinação. É importante esclarecer esta reação adversa (incomum) de forma a não criar dúvidas sobre os benefícios da vacinação.

CONTRIBUTO DOS AUTORES

JBR: Acompanhamento clínico, recursos.
MP: Redação do manuscrito, recursos, revisão crítica.
IA: Revisão crítica.
RS: Pesquisa bibliográfica, revisão crítica.

PROTEÇÃO DE PESSOAS E ANIMAIS

Os autores declaram que os procedimentos seguidos estavam de acordo com os regulamentos estabelecidos pelos responsáveis da Comissão de Investigação Clínica e Ética e de acordo com a Declaração de Helsínquia da Associação Médica Mundial atualizada em 2013.

CONFIDENCIALIDADE DOS DADOS

Os autores declaram ter seguido os protocolos do seu centro de trabalho acerca da publicação de dados.

CONSENTIMENTO DO DOENTE

Obtido.

CONFLITOS DE INTERESSE

Os autores declaram não ter conflitos de interesse relacionados com o presente trabalho.

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ACTA MÉDICA PORTUGUESA
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Infeção Humana pelo Vírus Monkeypox

Human Monkeypox

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Palavras-chave: Doenças Transmissíveis Emergentes; Monkeypox; Surtos de Doenças; Vírus da Varíola dos Macacos
Keywords: Communicable Diseases, Emerging; Disease Outbreaks; Monkeypox virus; Monkeypox

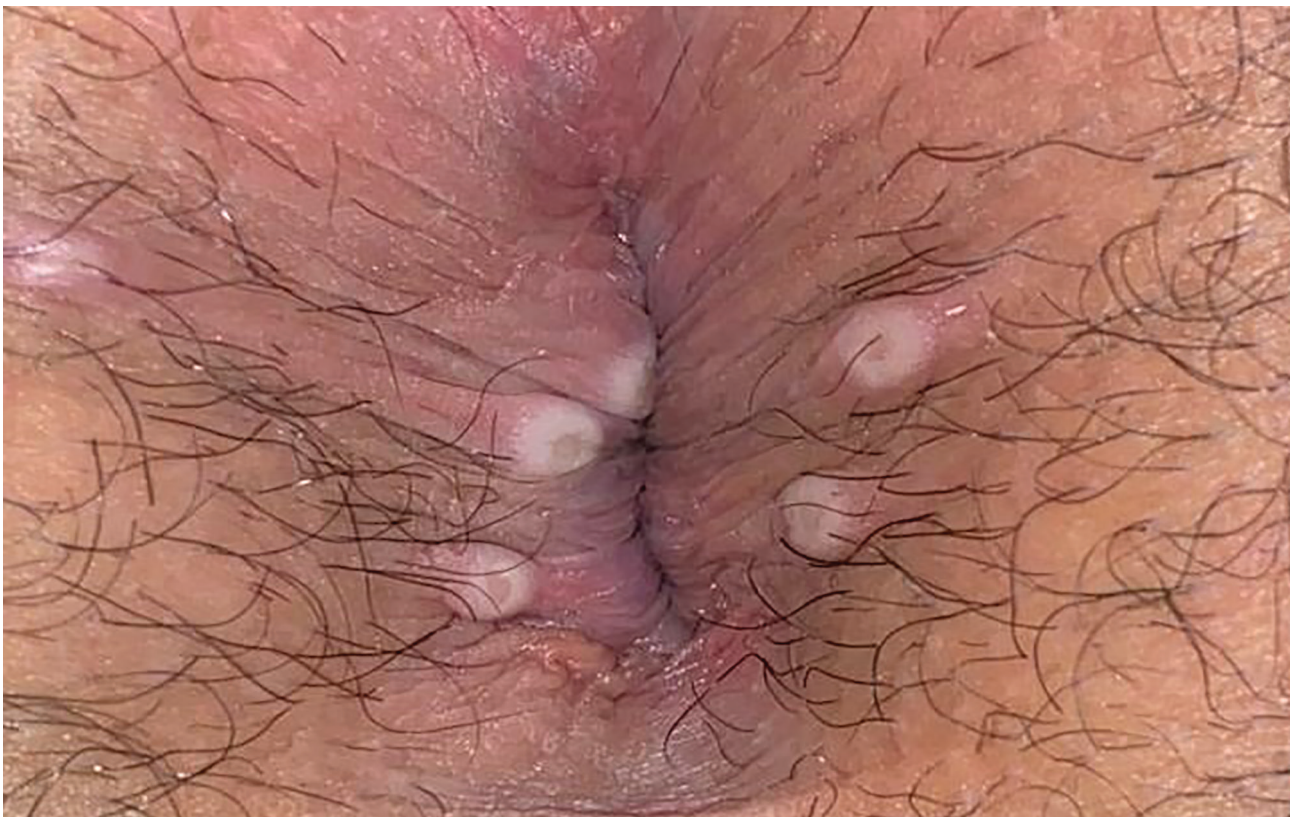

Figura 1 – Pápulas esbranquiçadas, umbilicadas, firmes, infracentimétricas, localizadas na região perianal

Um homem de 27 anos notou o aparecimento de lesões cutâneas assintomáticas na região perianal precedidas em um dia por cefaleias e febrícula. Reporta-se como homem que tem sexo com homens, com contactos com múltiplos parceiros no último mês. Nega viagens recentes ou contacto com animais. À observação, identificam-se pápulas esbranquiçadas localizadas na região perianal (Fig. 1) e adenopatias inguinais dolorosas. As amostras das lesões, colhidas por zaragatoa e enviadas para o Instituto Nacional de Saúde Dr. Ricardo Jorge, revelaram infeção pelo vírus *monkeypox* por PCR em tempo real. As lesões evoluíram com a formação de erosões cobertas por crosta central umbilicada e o quadro resolveu em duas semanas.

A infeção pelo vírus *monkeypox* (género orthopoxvírus) é uma zoonose viral endémica na África Central e Ocidental, cuja transmissão ocorre por contacto próximo com animais, pessoas infetadas ou material contaminado.[1,2] A transmissão interpessoal ocorre por contacto prolongado com lesões, fluídos corporais, gotículas respiratórias e materiais contaminados.[1,2]

CONTRIBUTO DOS AUTORES

JB: Planeamento e redação do manuscrito. Captação de imagens.

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MC: Redação do manuscrito. Captação de imagens.
CF: Orientação e supervisão do artigo.

PROTECÇÃO DE PESSOAS E ANIMAIS

Os autores declaram que os procedimentos seguidos estavam de acordo com os regulamentos estabelecidos pelos responsáveis da Comissão de Investigação Clínica e Ética e de acordo com a Declaração de Helsínquia da Associação Médica Mundial atualizada em 2013.

CONFIDENCIALIDADE DOS DADOS

Os autores declaram ter seguido os protocolos do seu centro de trabalho acerca da publicação de dados.

CONSENTIMENTO INFORMADO

Obtido.

CONFLITOS DE INTERESSE

Os autores declaram não ter qualquer conflito de interesse relativamente ao presente artigo.

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ACTA MÉDICA PORTUGUESA
A Revista Científica da Ordem dos Médicos

Recomendações do Grupo Português do Mieloma Múltiplo para Tratamento do Mieloma Múltiplo

Multiple Myeloma Treatment Guidelines by the Portuguese Group of Multiple Myeloma

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RESUMO

O tratamento do mieloma múltiplo tem sido amplamente alterado com introdução de várias terapêuticas inovadoras. A otimização da sequenciação terapêutica através do uso combinado dos vários fármacos desenvolvidos nos últimos anos e a atenção dada às características dos doentes têm permitido diminuir toxicidades e aumentar a sobrevivência dos doentes, bem como aumentar a sua qualidade de vida. As presentes recomendações terapêuticas do Grupo Português do Mieloma Múltiplo oferecem orientações para o tratamento de primeira linha e progressão/recaída. As recomendações são fundamentadas evidenciando os dados que justificam cada escolha e referindo os respetivos níveis de evidência que suportam essas opções. Sempre que possível é apresentado o respetivo enquadramento regulamentar nacional. Estas recomendações constituem um avanço para o melhor tratamento do mieloma múltiplo em Portugal.
Palavras-chave: Mieloma Múltiplo/tratamento farmacológico; Portugal

ABSTRACT

The treatment of multiple myeloma has profoundly changed with the introduction of several innovative therapies. The optimization of therapeutic sequencing through the combined use of the various drugs developed in recent years and the attention given to the characteristics of patients have allowed the reduction of toxicities and increased survival and quality of life of patients with multiple myeloma. These treatment recommendations from the Portuguese Multiple Myeloma Group offer guidance for first-line treatment and progression/relapse situations. These recommendations are given highlighting the data that justify each choice and referring to the respective levels of evidence that support these options. Whenever possible, the respective national regulatory framework is presented. These recommendations constitute an advance towards the best treatment of multiple myeloma in Portugal.
Keywords: Multiple Myeloma/drug therapy; Portugal

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INTRODUÇÃO

Ao longo dos últimos anos, o tratamento do mieloma múltiplo (MM) sofreu grandes avanços com a disponibilidade de várias opções terapêuticas quer para a primeira linha, quer para linhas subsequentes de tratamento. A sobrevivência livre de progressão (SLP) e a sobrevivência global (SG) dos doentes tem aumentado de forma considerável.[1,2] Vários grupos nacionais e internacionais de interesse em MM têm publicado recomendações que podem variar na sua aplicação na prática clínica nos diferentes países, como consequência de diferentes políticas de reembolso de fármacos em cada país.[3–6]

O Grupo Português do Mieloma Múltiplo (GPMM) apresenta aqui as suas recomendações para o tratamento do MM nas suas diferentes fases da doença. Além da apresentação de propostas para as várias linhas terapêuticas, são efetuadas considerações acerca da justificação de tais escolhas e da importância da adequada sequenciação de tratamento, com vista a atingimento doença residual mensurável (DRM) negativa, o que está associado a uma SG superior.[7] Para cada opção terapêutica são apresentados o nível e grau de evidência que suporta a recomendação do protocolo sugerido. Estas recomendações são apresentadas em texto completo no Apêndice 1 (Apêndice 1: https://www.actamedicaportuguesa.com/revista/index.php/amp/article/view/19037/15153).

O tratamento do MM adequa-se, em primeira mão, a dois grandes grupos de doentes: aqueles que, pela sua idade (≤ 70 anos), ausência de comorbilidades importantes e com robustez funcional são passíveis de suportar tratamentos intensivos como quimioterapia de alta dose seguida de transplante autólogo de progenitores hematopoiéticos (TAPH); e aqueles que, quer pela idade mais avançada (> 70 anos) quer pelas comorbilidades e outras fragilidades, não são candidatos a transplante.[8]

TRATAMENTO DE PRIMEIRA LINHA

Indução nos doentes elegíveis para transplante autólogo de progenitores hematopoiéticos

Os protocolos de indução pré-TAPH são baseados na combinação de bortezomib (V) e dexametasona (D),[9] ao qual se podem associar ciclofosfamida (VCD), talidomida (VTD) ou lenalidomida (VRD). Recentemente, foram publicados os resultados dos estudos que comparam os tripletos VTD e VRD com a sua associação a daratumumab (Dara).[10,11]

A única comparação direta entre VCD e VTD evidencia uma taxa de resposta superior a muito boa resposta parcial (MBRP) significativamente maior para os doentes tratados com VTD (66,3% *vs* 56,2%, $p = 0,05$).[12] A vantagem de VTD sobre VCD é corroborada por uma meta-análise.[13]

O ensaio clínico CASSIOPEIA, que comparou daratumumab, bortezomib-talidomida-dexametasona (DaraVTD) a VTD, demonstrou vantagem significativa do braço com DaraVTD, com uma redução do risco de progressão ou morte em 53%.[11] De igual modo, o ensaio clínico de fase 2 GRIFFIN, que compara VRD com daratumumab, bortezomib- lenalidomida- dexametasona (DaraVRD), mostrou vantagem para os doentes tratados com DaraVRD.[10]

Novas combinações, explorando a substituição de bortezomib pelo inibidor do proteassoma de segunda geração carfilzomib (K) (ensaio clínico FORTE),[14] ou a sua associação a anticorpos monoclonais anti-CD38 – DaraKRD (MASTER)[15] ou IsaKRD (GMMG-CONCEPT),[16] poderão vir a constituir alternativas promissoras como protocolos de indução pré-transplante.

No entanto, se nos restringirmos às combinações aprovadas pela Agência Europeia do Medicamento (EMA) e comparticipadas em Portugal, o tratamento com DaraVTD deve ser, atualmente, considerado preferencial (Evidência IA proveniente de revisões sistemáticas de ensaios clínicos controlados). Se esta terapêutica não puder ser realizada, os doentes poderão ser tratados com VTD (Evidência IA) ou VRD (Evidência IA). Com os dados atuais, a alternativa VCD (Evidência IIB proveniente de estudos de coorte e ensaios randomizados de menor qualidade) é a menos recomendada (Fig. 1).

O número de ciclos ideal pré-TAPH não é consensual. No entanto, não se recomenda mais de seis ciclos com tripletos (VTD, VRD, VCD) nem mais de quatro ciclos com o quadrupleto DaraVTD.

Transplante autólogo de progenitores hematopoiéticos

Apesar das mudanças na abordagem da primeira linha de tratamento do MM com a inclusão de esquemas terapêuticos mais eficazes e menos tóxicos, a quimioterapia de alta dose com melfalano seguida de TAPH mantém-se como tratamento *gold standard* nos doentes com MM elegíveis para transplante (Evidência IA).

São elegíveis para TAPH todos os doentes com MM recentemente diagnosticado com idade menor ou igual a 70 anos, com ECOG PS 0-2 e sem comorbilidades, nomeadamente cardíacas, pulmonares, hepáticas ou renais.[8,17] A idade cronológica não deve ser usada como único fator de elegibilidade para transplante dada a sua subjetividade.[18,19] O uso de *scores* procura eliminar esse viés e permite uma abordagem global do doente referenciado para TAPH.[20] A avaliação risco-benefício do TAPH deve ser discutida entre as equipas transplantadoras e referenciadoras.

Os doentes sem resposta parcial após indução apresentam SG inferior.[21–23] Deste modo, recomenda-se que a resposta deva ser aprofundada com outro esquema de segunda linha antes do TAPH.[22–24]

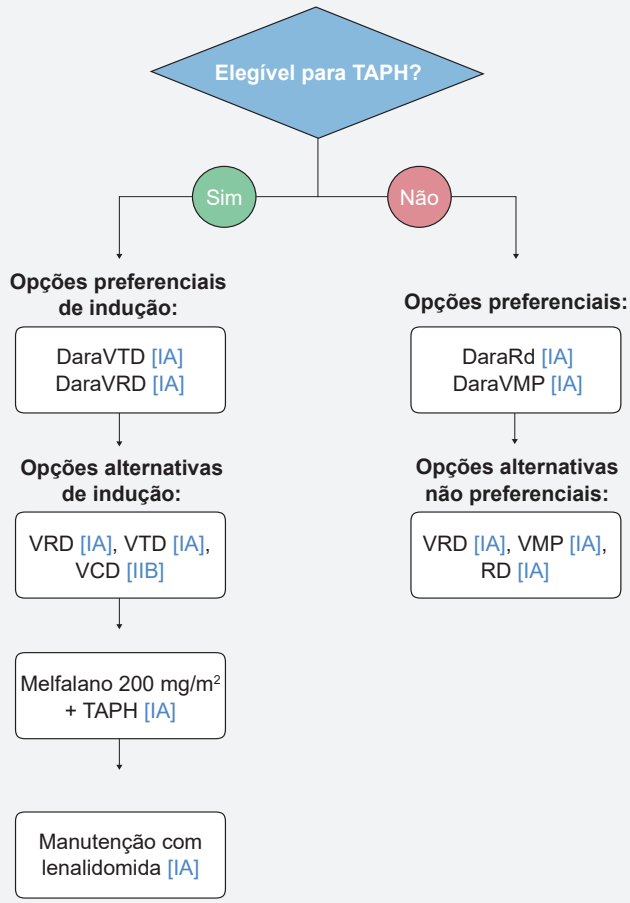

Figura 1 – Protocolos propostos para primeira linha de tratamento dos doentes elegíveis e não elegíveis para TAPH

A mobilização de progenitores hematopoiéticos é crucial no processo da transplantação autóloga destes doentes. Atendendo à necessidade de internamento e complicações infeciosas, entre outras, associadas a doses elevadas de ciclofosfamida (> 4 g/m^2), recomenda-se a mobilização baseada em *granulocyte colony stimulating factor* (G-CSF) isoladamente, recorrendo ao plerixafor quando necessário. Apesar de não existir efeito terapêutico comprovado, os doentes com resposta inferior a resposta completa poderão ser mobilizados em ambulatório com baixa dose de ciclofosfamida (1,5 g/m^2) e G-CSF.[25] Para assegurar um enxerto eficaz e célere após infusão, a quantidade de progenitores hematopoiéticos a serem colhidos para cada infusão deve ser de, no mínimo, 3 x 10^6 células CD34+/kg.[8,26,27] Recomenda-se que a mobilização ocorra no máximo após quatro a seis ciclos de indução com esquemas que incluam talidomida e após dois a quatro ciclos de indução com esquemas que incluam lenalidomida. Deve também haver um período de *washout* de imunomoduladores pré-mobilização, respetivamente duas a quatro semanas para talidomida e quatro a seis semanas para lenalidomida.[26,27]

O regime de condicionamento assenta em melfalano 200 mg/m^2 como *standard*. Nos doentes com creatinina > 2 mg/dL ou clearance de creatinina < 30 mL/min/1.73 m^2, a dose deve ser reduzida para 140 mg/m^2.[28] Doentes com lesão renal e necessidade de diálise não devem ser privados da realização do TAPH, sendo a dose recomendada nestes doentes de 200 mg/m^2.[28] Também não existe indicação formal para redução de dose nos doentes entre os 65 e os 70 anos.[8,29]

CONSOLIDAÇÃO APÓS TAPH

A terapêutica de consolidação refere-se a dois a três ciclos de um regime igual ou mais eficiente que aquele utilizado na indução pré-TAPH, com o objetivo de aprofundar a resposta no sentido de aumentar a probabilidade de obtenção de DRM negativa. A consolidação deve ter sempre em consideração o número de ciclos realizados na indução e a toxicidade existente. O recurso a transplante em *tandem* é, desta forma e quando possível, uma consolidação da resposta obtida no primeiro transplante.

O estudo EMN02/HO95, com uma mediana de seguimento de 42 meses, mostrou que a consolidação com ciclos de VRD melhorava a sobrevivência livre de progressão mediana (SLPm)quando comparada com o braço sem consolidação (58,9 *vs* 45,5 meses; *p* = 0,014).[30] De notar que o esquema de indução neste estudo foi de apenas quatro ciclos de VCD (Evidência IIB).

A estratégia da realização de um segundo transplante até ao máximo de seis meses após o primeiro, conhecido como *tandem*, continua a apresentar resultados contraditórios apesar de largamente explorada. O benefício foi observado principalmente em doentes de elevado risco citogenético (*p* = 0,042).[31]

Por outro lado, o estudo StaMINA não mostra vantagem do *tandem* nos doentes com MM à exceção dos doentes com citogenética de alto risco com SLP aos seis anos de 43,6% para o *tandem versus* 26% para o TAPH único (*p* = 0,03).[31]

Assim, recomenda-se que o transplante em *tandem* seja oferecido a todos os doentes com características de alto risco, nomeadamente citogenética com t(4;14) ± t(14;16) ± del(17p) ± ganho(1q21), refratariedade primária à indução e em doentes que após o primeiro TAPH não atinjam uma resposta ótima, nomeadamente maior a muito boa resposta parcial (MBRP) (Evidência IB resultado de ensaios clínicos controlados e randomizados).[30]

MANUTENÇÃO APÓS TAPH

O tratamento de manutenção após TAPH aprovado pela EMA e comparticipado em Portugal centra-se no tratamento com lenalidomida (Evidência IA), até progressão/recaída

ou toxicidade inaceitável.[32] Deve ser iniciada após recuperação hematopoiética e/ou confirmação de resposta ao TAPH, idealmente ao dia +100. A manutenção com outros fármacos – bortezomib, carfilzomib e daratumumab – não foi ainda aprovada pela EMA apesar da evidência existente em alguns ensaios clínicos.[33]

A manutenção com lenalidomida após TAPH mostra benefícios significativos em termos de SLP e SG obtidos em dois grandes ensaios clínicos de fase 3, randomizados *versus* placebo.[32,34] Além disso, uma meta-análise que incluiu mais de 1200 doentes, com mediana de seguimento de 79,5 meses, confirmou que a manutenção com lenalidomida após TAPH aumentava a SLPm em mais dois anos e a SG em mais dois anos e meio *versus* placebo.[35]

A manutenção com inibição do proteassoma (IP) como bortezomib (V), carfilzomib (K) e ixazomib (Ixa) tem sido estudada em ensaios clínicos de fases 2 e 3.[36–38] Um ensaio recente de fase III comparou ixazomib (Ixa) oral com placebo após TAPH (Evidência IA) e mostrou vantagem na SLPm para manutenção com ixazomib *versus* placebo, mas sem vantagem de sobrevivência global.[39] A manutenção com bortezomib mostrou vantagem de SLP comparada com talidomida num ensaio clínico onde a terapêutica de indução dos dois ramos do ensaio foi também diferente (Evidência IB).[40–42]

Relativamente aos doentes com MM de alto risco citogenético existe Evidência IIB na vantagem de utilização conjunta de lenalidomida e bortezomib no tratamento de manutenção.[43]

TRATAMENTO DE PRIMEIRA LINHA DOS DOENTES NÃO ELEGÍVEIS PARA TAPH

Nesta população recomenda-se, por ordem preferencial, a realização dos seguintes protocolos terapêuticos Dara-Rd (daratumumab, lenalidomida, dexametasona), Dara-VMP (daratumumab, bortezomib, melfalano) e VRd (bortezomib, lenalidomida e dexametasona), todos com Evidência IA (Fig. 1). Em Portugal, a utilização de daratumumab foi recentemente comparticipada em primeira linha para doentes não elegíveis para TAPH. A associação Dara-Rd foi aprovada em primeira linha em todos os doentes não elegíveis para TAPH, enquanto a associação Dara-VMP apenas para a subpopulação de doentes sem alterações citogenéticas de alto risco. O esquema VRd encontra-se aprovado pela EMA, mas não comparticipado em Portugal. Nestes doentes, a adição de anticorpos monoclonais anti-CD38 aos esquemas de tratamento *standard* de Rd e VMP traduziu-se numa maior eficácia relativamente aos mesmos, quer em termos de SLP e SG.[44,45]

TRATAMENTO DA PRIMEIRA RECAÍDA

O tratamento proposto após a primeira recaída deve possibilitar o resgate do maior número de doentes e deve ser escolhido criteriosamente entre as opções disponíveis, de modo a aumentar a SLP e a SG dos doentes, mantendo e até promovendo a melhoria da qualidade de vida. Dados de vida real publicados sugerem que apenas 32% a 61% dos doentes recebem uma segunda linha, e 14% a 38% recebem uma terceira linha terapêutica.[46–49] É importante considerar o início do tratamento em situações de recaída bioquímica em casos de MM de alto risco, ou com cinética de recaída acelerada e/ou agressiva.

Em Portugal, vários são os regimes aprovados e reembolsados para utilização em segunda linha (Fig. 2).

Assim, recomendamos esquemas de segunda linha a serem considerados após cada um dos esquemas terapêuticos passíveis de terem sido realizados em primeira linha, quer previamente a 2022 quer após aprovação de primeira

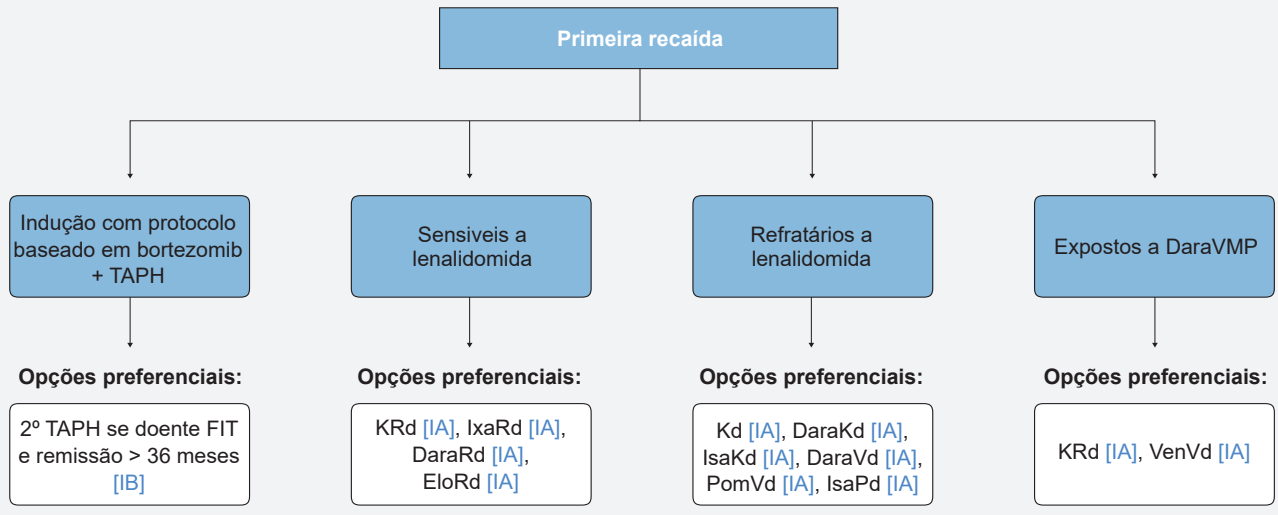

Figura 2 – Protocolos propostos para primeira recaída baseados nos esquemas anteriormente realizados

linha com daratumumab. Dada a heterogeneidade clonal e processos de resistência que se estabelecem durante os tratamentos, a otimização da segunda linha deve basear-se na escolha de grupos terapêuticos diferentes dos utilizados na linha anterior e deve considerar a fragilidade do doente, as suas comorbilidades e eventuais toxicidades que tenha existido, e eventualmente mantido, a fármacos utilizados. Para além destas considerações, os doentes de alto e muito alto risco, ou seja, doentes com uma ou mais alterações citogenéticas de mau prognóstico, doença extramedular, Revised International Staging Score (R-ISS) III ou doentes com tempo de SLP após primeira linha otimizada inferior a 12 meses, devem ser propostos para esquemas mais eficientes como tripletos com daratumumab (D) ou carfilzomib (K) ou pomalidomida (P), se tiverem a robustez física necessária. O tratamento da primeira recaída deve ser o mais otimizado possível, dada a maior probabilidade de se conseguir ainda aumentar a sobrevivência dos doentes, quando comparamos com resultados obtidos com tratamentos de recaídas posteriores.[50–53]

Doentes previamente tratados com tripleto incluindo bortezomib e quimioterapia de alta-dose seguida de TAPH, sem comorbilidades significativas e com remissão duradoura (≥ 36 meses), poderão ser candidatos a re-indução com esquemas com bortezomib seguido de um segundo TAPH. (Evidência IB).[50–52]

Nos doentes em que um segundo TAPH não seja opção e não haja refratariedade à lenalidomida, é consensual que o tratamento de segunda linha se baseie num tripleto que inclua Rd, estando aprovados em Portugal os regimes KRd, IxaRd, DaraRd e elotuzumabRd (EloRd). Não existem comparações diretas entre estes regimes, mas todos demonstraram superioridade de SLPm em relação a Rd (Evidência IA).[53–56]

Nos doentes em que a primeira linha de indução incluiu Rd e naqueles que progrediram sob lenalidomida na dose de 10 mg/d como tratamento de manutenção após TAPH, o tratamento da primeira recaída deverá, se possível, substituir a lenalidomida por um IP (carfilzomib ou bortezomib) ou por um imunomodulador de terceira geração (pomalidomida), As combinações aprovadas em Portugal em primeira recaída de doença com eficácia demonstrada em doentes previamente expostos/refratários a lenalidomida são as seguintes: carfilzomib, dexametasona (Kd), daratumumab, carfilzomib, dexametasona (DaraKd), isatuximab, carfilzomib e dexametasona (IsaKd), daratumumab, bortezomib, dexametasona (DaraVd) e pomalidomida, bortezomib, dexametasona (PVd) (Evidência IA).[57]

TRATAMENTO DA SEGUNDA RECAÍDA E POSTERIORES

Considerando as recentes mudanças na primeira linha e primeira recaída, a abordagem do MM em recaída e/ou refractário (MMrr) após duas ou mais linhas de tratamento, é um dos maiores desafios no tratamento destes doentes. Doentes refratários a dois inibidores de proteassomas, dois imunomoduladores e anticorpos monoclonais anti-CD38 apresentam mau prognóstico com sobrevivência global mediana com sobrevivência global mediana (SGm) de 5,6 meses.[57] A inclusão de um doente com MMrr em ensaio clínico deve ser sempre promovida.

Para os doentes expostos ou refratários ao bortezomib e à lenalidomida sem exposição prévia a anticorpos monoclonais anti-CD38, recomendam-se preferencialmente, esquemas baseados em anticorpos monoclonais como os protocolos de fase 3 DaraKd, IsaKd, DaraPd e IsaPd (Evidência IA). O protocolo de fase 2 com EloPd (Evidência IIB) deve também ser considerada uma opção (Fig. 3) ainda não comparticipada em Portugal.

As outras opções baseadas em anticorpos monoclonais anti-CD38 a serem consideradas nestes doentes assentam na pomalidomida como backbone. O estudo APOLLO mostra benefício para DaraPd sobre Pd com SLPm de 12,4 meses *versus* 6,0 meses [HR 0,63 (IC 95% 0,47 - 0,85), log-rank p < 0,0018 (Evidência IIB)].[58] Do mesmo modo, o estudo de fase 3 ICARIA mostra vantagem para isatuximab-pomalidomida-dexametasona (IsaPd) *versus* Pd também em doentes previamente tratados com duas ou mais linhas de terapêutica, incluindo lenalidomida e um inibidor de proteassoma (Evidência IA).[59,60]

Para os doentes tripla ou penta-refratários, as opções terapêuticas passam pelo uso de agentes com novos mecanismos de ação, sendo o selinexor, um inibidor seletivo de exportação nuclear, e o belantamab mafodotina, um anticorpo monoclonal conjugado com monometilauristatina F anti-B cell maturation antigen (BMCA), opções a considerar em monoterapia (Evidência IIB).

A quimioterapia convencional pode ser uma opção para doentes com mieloma múltiplo recaído e refratário (MMrr), principalmente como terapia de resgate e ponte para outras abordagens terapêuticas (Evidência IV).[61] Outras opções terapêuticas de resgate temporárias como a monoterapia com alta dose de ciclofosfamida ou bendamustina podem ser exploradas em doentes com MMrr altamente tratados e expostos a múltiplos agentes.[62,63]

NOVAS TERAPÊUTICAS EM MIELOMA

Apesar dos significativos ganhos clínicos obtidos nos últimos anos, o tratamento do MM continua a ter áreas de óbvia necessidade clínica como a doença multirrefratária, a doença extramedular ou o mau prognóstico citogenético/molecular. De entre as terapêuticas que se espera poderem chegar à prática clínica nos próximos anos encontram-se as CAR-Ts (terapias celulares com recetor de

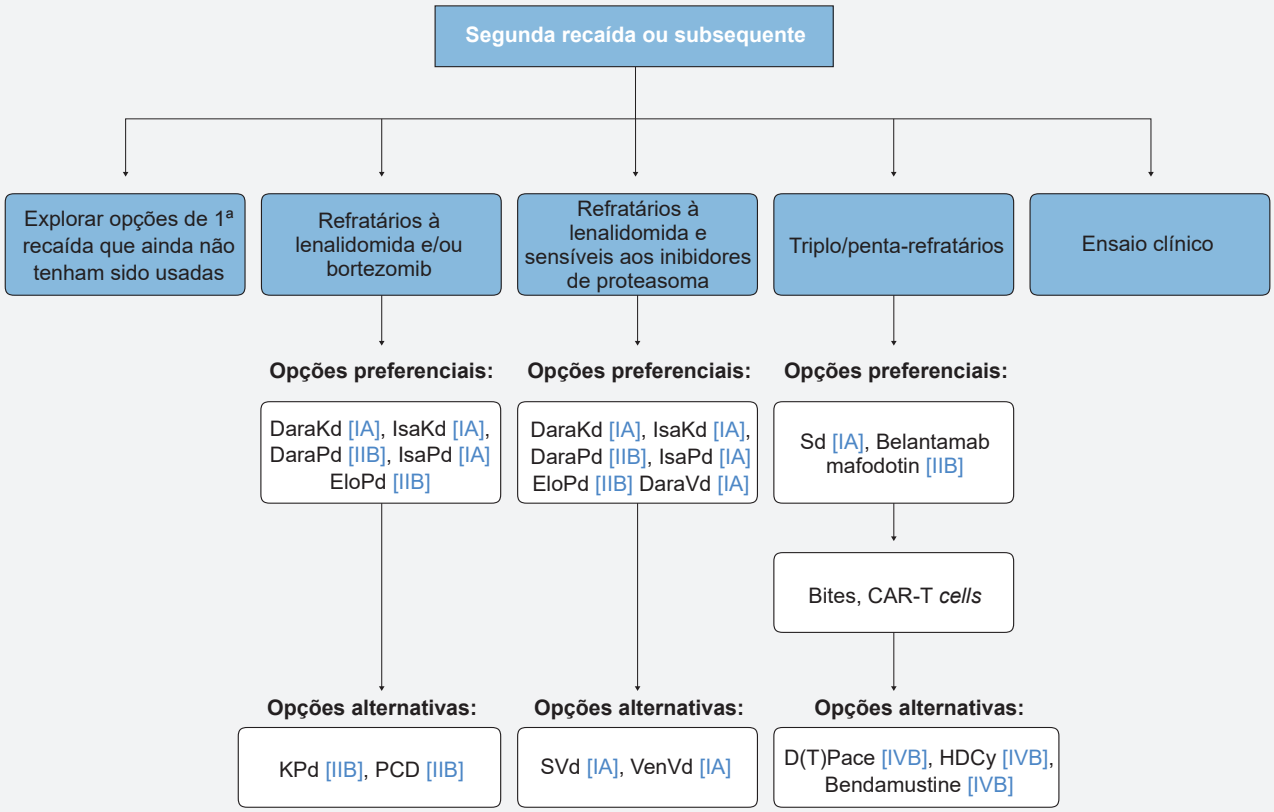

Figura 3 – Protocolos propostos para segunda recaída ou subsequentes baseados nos esquemas anteriormente realizados

antigénio quimérico), os anticorpos bi-específicos, os anticorpos monoclonais conjugados com fármacos anticancerígenos, peptídeos conjugados com fármacos, como o melflufeno (*melphalan flufenamide*) e os moduladores da cereblon E3 ligase (CELMoDs).

MODULADORES DA LIGASE E3 DO CEREBLON (CELMoDs)

Os moduladores da cereblon E3 ligase representam a evolução dos agentes imunomoduladores, com maior afinidade para a cereblon e, por isso, têm o potencial de exercer um maior efeito imunoestimulante e anti-tumoral. A iberdomida é um novo CELMoD, com estudos de fase 3 a decorrer internacionalmente e com resultados em fase 2 muito promissores.[65] A mezigdomida foi avaliada num ensaio de fase 1 em associação com dexametasona em 76 doentes triplo-refratários, demonstrando uma taxa de resposta de 48%.[64–66]

TERAPIAS CELULARES

Muitas das terapias celulares em investigação em fase clínica têm como alvo o antigénio BCMA, uma glicoproteína transmembranar solúvel que faz parte da superfamília do recetor do Factor de Necrose Tumoral (TNFRSF17) e que está sobre-expressa em células B maduras e plasmócitos. O idecabtagene vicleucel (Ide-cel) já tem aprovação pela EMA e pela Food and Drug Administration (FDA), e o ciltacabtagene autoleucel (Cilta-cel) já tem aprovação pela EMA.[67,68] Os dados de segurança mostram que as toxicidades mais frequentes são hematológicas (incidência de citopenias até 100%) e infeciosas. Quanto a eventos adversos específicos para CAR-T, os dados são muito favoráveis, com taxas de síndrome de libertação de citoquinas (*CRS*) de graus três a cinco inferior a 10% e taxas de toxicidade neurológica de graus três a cinco em menos de 5% dos doentes.

ANTICORPOS BI-ESPECÍFICOS

À semelhança das CAR-T, os anticorpos bi-específicos (BiTES) usam o potencial anti-plasmocitário dos linfócitos autólogos, mas apresentam a potencial vantagem de permitir uma disponibilidade imediata (*off-the-shelf*). Existem pelo menos seis destas moléculas em estudo clínico, a maioria em fase 1, todos em doentes triplo-refratários, em que um anti-BCMA/anti-CD3 (teclistamab) está já aprovado pela EMA.[70]

CONJUGADOS ANTICORPO-FÁRMACO

A conjugação de anticorpos monoclonais anti-BCMA ou anti-CD38 com fármacos citotóxicos tem por objetivo associar a seletividade do anticorpo monoclonal, e a sua capacidade de despoletar uma resposta imune, com o efeito apotótico do citotóxico sob a célula maligna.[71]

Além do belantamab mafodotin, foram recentemente apresentados dados promissores com modakafusp alfa, uma imunocitoquina que conjuga um anticorpo monoclonal anti-CD38 com duas moléculas de IFNa2b.[72]

NOTAS FINAIS

O tratamento do mieloma múltiplo está em constante mudança. A otimização da sequenciação terapêutica através do uso combinado dos vários fármacos desenvolvidos nos últimos anos, e a atenção dada às características dos doentes no que diz respeito à robustez funcional e a comorbilidades presentes, têm permitido minimizar toxicidade, aumentar a sobrevivência livre de progressão, a sobrevivência global, e a qualidade de vida dos doentes com mieloma múltiplo. Estas recomendações do Grupo Português do Mieloma Múltiplo oferecem orientações chave no tratamento de primeira linha e progressão/recaída, enfatizando-se a necessidade de tratar os doentes com a melhor terapêutica possível em cada fase da doença fornecendo dados que o justifiquem e níveis de evidência que suportem essas opções, enquadrando-as sempre que possível a nível regulamentar. Constituem, assim, um avanço para o melhor tratamento do mieloma múltiplo em Portugal.

CONTRIBUTO DOS AUTORES

CJ, RB, CG: Conceção, desenho, escrita e revisão do artigo.

JS, CA, PB, HC, CC, GE, JGF, RG, AJ, AMa, AMo, MN, CLP, SP, AP, PS, HS, MPS, AT, FT, ABS, PL: Escrita e revisão do artigo.

PROTEÇÃO DE PESSOAS E ANIMAIS

Os autores declaram que os procedimentos seguidos estavam de acordo com os regulamentos estabelecidos pelos responsáveis da Comissão de Investigação Clínica e Ética e de acordo com a Declaração de Helsínquia da Associação Médica Mundial atualizada em 2013.

CONFLITOS DE INTERESSE

CJ recebeu honorários de consultadoria de BMS, Janssen, Amgen e Takeda; recebeu pagamentos ou honorários para palestras, apresentações, escrita de manuscritos ou eventos educacionais de BMS, Janssen, Amgen, GSK, Pfizer e Takeda; recebeu apoio de Janssen, Takeda e BMS para comparecer em reuniões/viagens; participou em conselhos de monitorização de segurança de dados ou conselhos consultivos para as entidades BMS, Janssen, Amgen, Pfizer e Takeda.

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HC recebeu apoio de Janssen para o pagamento da inscrição e hotel para a reunião anual da Sociedade Portuguesa de Hematologia.

GE recebeu pagamentos ou honorários para palestras, apresentações, escrita de manuscritos ou eventos educacionais de Takeda, Janssen e Amgen; recebeu apoio de Janssen para comparecer em reuniões/viagens; participou em conselhos de monitorização de segurança de dados ou conselhos consultivos para as entidades Janssen, Sanofi e GSK.

JGF recebeu apoio de Takeda para comparecer na *International Myeloma Society Annual Meeting* 2022.

AJ recebeu pagamentos ou honorários para palestras, apresentações, escrita de manuscritos ou eventos educacionais de Janssen, Amgen, Roche e Celgene; recebeu apoio de Celgene e Roche para comparecer em reuniões/viagens.

AMa recebeu apoio de Janssen para comparecer no congresso anual da European Hematology Association (EHA) 2021.

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SP recebeu pagamentos ou honorários para palestras,

apresentações, escrita de manuscritos ou eventos educacionais de Takeda; recebeu apoio de Takeda, Amgen Janssen e Sanofi para comparecer em reuniões/viagens; participou em conselhos de monitorização de segurança de dados ou conselhos consultivos para a entidade Janssen.

AR recebeu honorários de consultadoria de Prothena, Takeda, Roche; recebeu pagamentos ou honorários para palestras, apresentações, escrita de manuscritos ou eventos educacionais de Janssen, Roche, Takeda, Sanofi e Abbvie; recebeu apoio de Janssen, Pfizer e Amgen para comparecer em reuniões/viagens.

HMS recebeu pagamentos ou honorários para palestras, apresentações, escrita de manuscritos ou eventos educacionais de Amgen; recebeu pagamentos por prova pericial de Takeda; recebeu apoio de Abbvie, Amgen, Janssen e Gilead para comparecer em reuniões/viagens; participou em conselhos de monitorização de segurança de dados ou conselhos consultivos para as entidades Pfizer, Amgen e Jassen.

MPS recebeu apoio de Janssen, Amgen e Takeda para comparecer em reuniões/viagens.

PL recebeu honorários de consultadoria de Janssen, Takeda, Amgen, Novartis e BMS; recebeu pagamentos ou honorários para palestras, apresentações, escrita de manuscritos ou eventos educacionais de Amgen e Janssen; participou em conselhos de monitorização de segurança de dados ou conselhos consultivos para as entidades Amgen, Abbvie e Janssen.

CG recebeu pagamentos ou honorários para palestras, apresentações, escrita de manuscritos ou eventos educacionais de Takeda, Celgene, BMS, Janssen, Amgen, Sanofi e Gilead; recebeu apoio de Takeda, Celgene, BMS, Janssen e Amgen para comparecer em reuniões/viagens; participou em conselhos consultivos para as entidades Takeda, Celgene, BMS, Janssen, Amgen, Sanofi, Gilead e Pfizer.

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Airway Fibroepithelial Polyp: An Incidental Bronchoscopic Finding

Pólipo Fibroepitelial: Um Achado Broncoscópico Incidental

Keywords: Bronchial Neoplasms/diagnosis; Neoplasms, Fibroepithelial/diagnosis; Polyps/diagnosis
Palavras-chave: Neoplasias dos Bronquios/diagnóstico; Neoplasias Fibroepiteliais/diagnóstico; Pólipos/diagnóstico

Dear Editor,

Benign tracheobronchial tumors account for only 1.9% of pulmonary tumors[1] and airway fibroepithelial polyps are even rarer.

The authors describe a case of a 73-year-old man, former smoker, that presented with dry cough lasting several weeks. There was no previously known history of asthma, chronic obstructive lung disease, sinusitis, rhinitis, bronchiectasis, gastroesophageal reflux disease, cardiac, respiratory infections or other respiratory conditions. In addition, the patient was not taking any previous medicines and lung function tests were normal.

On chest computed tomography (CT) no changes were observed but since symptoms progressed a bronchoscopy was performed. At bronchoscopic examination, a 1.5 cm pedunculated lobulated endoluminal lesion with a glossy surface and hard consistency was arising from the anterior wall of the proximal portion of the trachea and resulting in luminal narrowing (Figs. 1A, 1B). The lesion was biopsied and fully removed with Diode Laser during a rigid bronchoscopy (Fig. 1C). At gross examination, it measured 1.2 x 1.0 cm with a lobulated contour and a glossy surface (Figs. 1A, 1B). Histopathologically, it had lobulated contours with marked papillary projections and consisted of fibrovascular stroma covered by normal respiratory epithelium (Fig. 1D).

A recent review of the literature found only 24 reported cases.[2] Intratracheal tumors may be asymptomatic but can present with wheezing, cough or dyspnea.[3] Size and location vary widely, and most cases are in located in the right bronchial tree.[3]

The etiology remains unclear, but it is thought to be associated with chronic inflammatory processes. There are reports of cases associated with smoking, asthma, thermal injury and foreign body aspiration.[4]

Histologically, it consists of fibrovascular stroma covered by normal respiratory or squamous epithelium, and

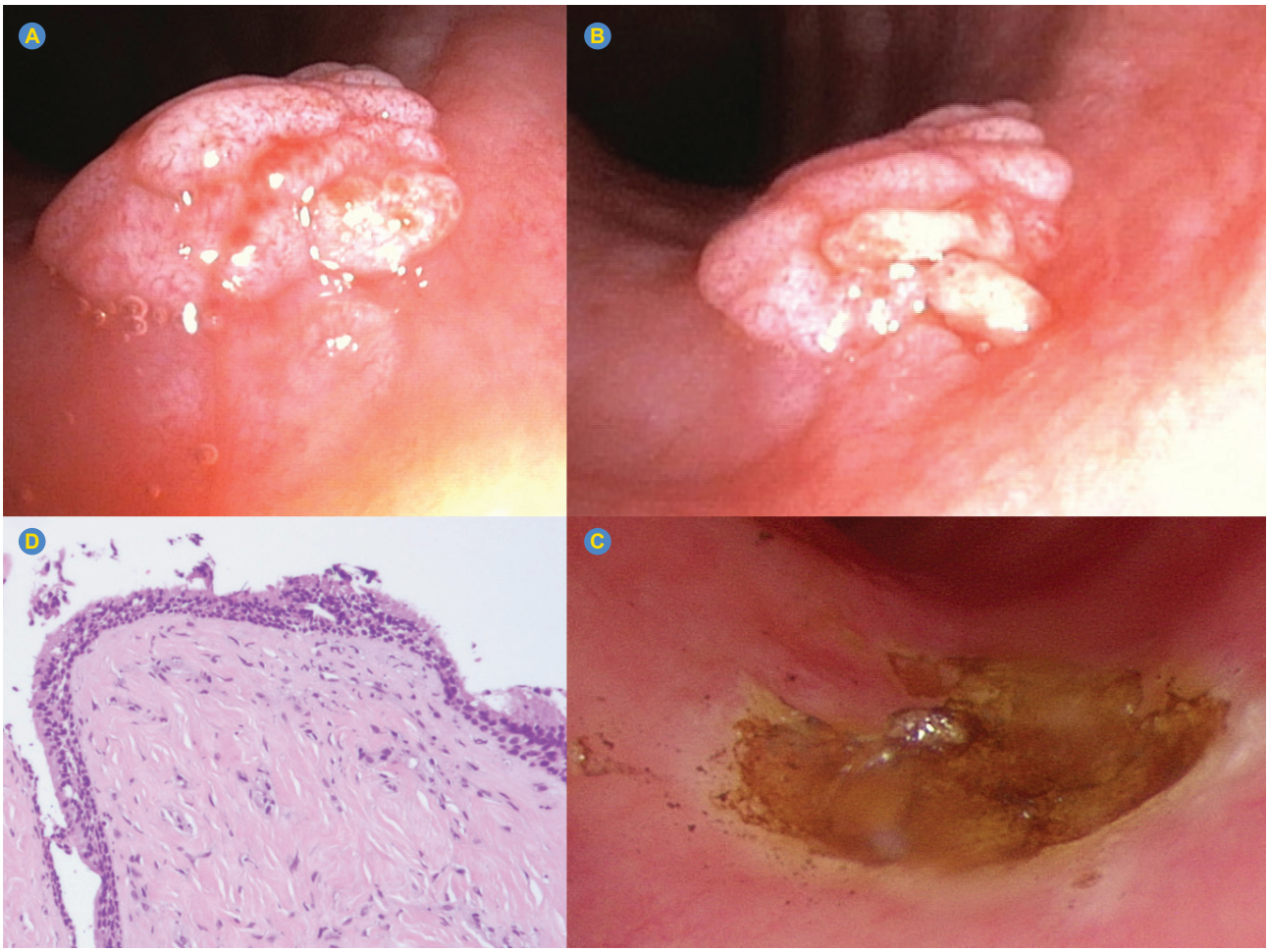

Figure 1 – Lobulated endoluminal lesion with a glossy surface (A, B); Resection of the polyp with laser (C); Lobulated contours with marked papillary projections consisting of fibrovascular stroma covered by normal respiratory epithelium (D).

macroscopically many are lobulated, resembling a blackberry.[5] Fibroepithelial polyps are histologically different from papillomas, which are related with human papilloma virus, affect mainly the vocal cords and trachea and have malignant potential.[3]

There is no consensus on the best treatment approach. Some authors advocate that small scarce symptomatic lesions should be treated with corticosteroids and antibiotics.[2] However, the treatment of choice may be endobronchial resection through mechanical debulking, laser or electrocautery. Surgery is rarely necessary and is an option when endobronchial resection is difficult or when pathological findings are controversial.[2]

We have not found any reports on recurrence or malignant transformation.[6]

The fibroepithelial polyp is a rare benign tumor that should be included in the differential diagnosis of all tracheobronchial tree lesions, especially in smokers and among patients with chronic obstructive pulmonary disease.[1]

AUTHOR CONTRIBUTIONS

MJS: Writing of the manuscript.

JNM: Writing of the manuscript.

SF: Review of the final version of the article.

PROTECTION OF HUMANS AND ANIMALS

The authors declare that the procedures were followed according to the regulations established by the Clinical Research and Ethics Committee and to the Helsinki Declaration of the World Medical Association updated in 2013.

DATA CONFIDENTIALITY

The authors declare having followed the protocols in use at their working center regarding patients' data publication.

PATIENT CONSENT

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Prison Mental Health in Portugal: Letter to the Editor about the WHO Status Report on Prison Health

Saúde Mental no Meio Prisional Português: Carta ao Editor acerca do Relatório sobre Saúde Prisional da OMS

Keywords: Correctional Facilities; Forensic Psychiatry; Health Policy; Mental Disorders; Portugal; Right to Health
Palavras-chave: Direito à Saúde; Estabelecimentos Correcionais; Perturbação Mental; Política de Saúde; Portugal; Psiquiatria Forense

To the Editor,

Prison health has received significant attention following the launch of the World Health Organization's Status Report on Prison Health in the WHO European Region 2022 last February.[1] The Status Report (SR) recognizes several health issues in the prison systems across Europe, such as overcrowding, limited access to hepatitis C treatment, and poor continuity of care.[1] It identifies mental health disorders (MHD) as the primary cause of morbidity in European prisons, with an estimated prevalence rate of 32.8%, compared with 13.1% in the general community.[1] Additionally, the report classifies suicide as the leading cause of death within prisons.[1] After discussing general considerations, the SR examines the prison health care scenario in each country. The document coincides with several concerns being raised about the quality of health care in Portuguese prisons, specifically regarding mental health (MH).[2]

Several misconceptions may hinder the recruitment of psychiatrists to work in prisons. These include the beliefs that incarcerated patients are less deserving of mental health care, that prison psychiatry supports mass incarceration and that prison health care environment is more prone to safety risks to psychiatrists than other settings.[3] Despite these difficulties, Portuguese authorities reported 19 psychiatrists working in prisons at full-time equivalent (FTE) — a ratio of 1.7 psychiatrists for every 1000 incarcerated individuals, compared to 0.1 for the general population.[1] We should, nonetheless, express our reservations about the reported availability. Since one FTE corresponds to roughly 40 hours of weekly work and given that there are psychiatrists that operate as external providers, it seems unlikely that all the 19 psychiatrists work on FTE. We believe a more accurate measurement of resource availability should be based on the number of actual work hours psychiatrists perform at each prison facility. Nonetheless, the favourable ratio of psychiatrists to inmates raises some perplexities when considering the SR findings. Specifically, the scarcity of data provided by the Portuguese prison system about MH appears to contradict the reported availability of psychiatric care. The SR section regarding Portugal does not include records on the number of individuals living in prison diagnosed with MHD. Similarly, there are no records of the number of people diagnosed with MHD that received or completed treatment. Without thorough data, the main contribution of the SR concerning Portugal is highlighting how much work needs to be done to improve the organization and effectiveness of prison MH interventions.

Portugal should prioritize prison MH policies for several reasons. First, there is a duty to provide equivalence of care to ensure that incarcerated individuals maintain other fundamental rights, such as the right to health. Second, incarcerated individuals with MHD are particularly vulnerable, with higher risk of suicide, violence, and victimisation.[4] Third, although prisons may be an unfortunate outcome for individuals with severe mental illness — often lacking insight into their illness — and who could not be reached by conventional community-based health care providers, they may also provide an opportunity to intervene effectively.[5] Consequentially, a well prepared prison health structure is fundamental. Finally, there is compelling evidence that MHD treatment can lead to decreased rates of repeat offending.[5] Therefore, addressing prison MH is both an individual and public health matter requiring urgent attention.

COMPETING INTERESTS

The author have declared that no competing interests exist.

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Case Report of a Challenging Diagnosis of Nasal Tuberculosis

Caso Clínico de um Diagnóstico Desafiante de Tuberculose Nasal

Keywords: Antitubercular Agents/therapeutic use; Nose Diseases/drug therapy; Tuberculosis/diagnosis; Tuberculosis/drug therapy
Palavras-chave: Antituberculosos/uso terapêutico; Doenças Nasais/tratamento farmacológico; Tuberculose/diagnóstico; Tuberculose/tratamento farmacológico

Dear Editor,

The incidence of tuberculosis (TB) has been steadily declining for the past two decades. However, since the beginning of the COVID-19 pandemic, the TB incidence rate is estimated to have increased.[1]

Although western Europe is considered a low TB incidence region, recent migration trends have called attention to its ongoing relevance, since ear, nose, and throat (ENT) manifestations, such as nasal and nasopharyngeal tuberculosis, are rare.[2]

We describe a clinical case of a 77-year-old white man presenting to the ENT clinic with a two-week history of right nasal obstruction and localized pain. Anterior rhinoscopy revealed a friable, erythematous crusting of the anterior right septal mucosa. A biopsy was done showing inflammatory exudate, ulceration, and no evidence of malignancy. The patient started oral antibiotics and corticosteroids, with limited improvement.

At re-assessment, an anterior septal friable perforation was seen, and a paranasal sinus computed tomography (CT) scan revealed thickening of the nasal vestibule and anterior nasal septum with irregularity of the right mucosa and septal perforation. Paranasal sinuses were clear and pneumatized. No bone lesions were found. Punch biopsy of the septal ulcer was repeated, confirming the ulcerative granulomatous lesion with caseating necrosis without vasculitic nor neoplasic features (Fig. 1A). No microorganisms were found.

An immune panel, a klebsiella nasal swab and an HIV test were negative.

At this point, a review of the patient's medical history disclosed an episode of ganglionar tuberculosis 30 years ago. The patient denied fever, weight loss or night sweats and chest CT was normal. The histopathologic review of the tissue sample taken previously was required and staining with Ziehl-Neelsen was positive for acid-alcohol resistant bacilli. The polimerase chain reaction (PCR) and culture test were positive for Mycobacterium tuberculosis (Fig. 1B).

The patient started antitubercular therapy with isoniazid, rifampin, ethambutol and pyrazinamide for four months, followed by two months of isoniazid and rifampin.

At the first- evaluation one month following treatment, the patient was clinically improved. Anterior septal perforation remained, which resulted in nasal tip ptosis.

Nasal tuberculosis is a rare entity, and clinical presentation with ulcerative and destructive features can mimic malignancy, emphasizing not only the need for biopsy, but also routine microbiology and appropriate culture tests, so that infectious causes are not overlooked, and treatment is not delayed.

The diagnosis is difficult, especially because it requires a high diagnostic suspicion and histological confirmation is hampered by lengthy and false negative results. In fact, smears of acid-fast bacilli and cultures tend to be negative in nasal tuberculosis.

Given the recent reversal in TB prevalence trends worldwide, physicians should bear in mind that, even though nasal tuberculosis is a challenging diagnosis, new diagnostic tools such as PCR or interferon-γ assay can be extremely useful to achieve prompt results.[3]

AUTHOR CONTRIBUTIONS

JID: Conception and writing of the manuscript.
ANP: Writing and critical review of the manuscript.
FSV: Data collection.
SSC, LM: Critical review of the manuscript.

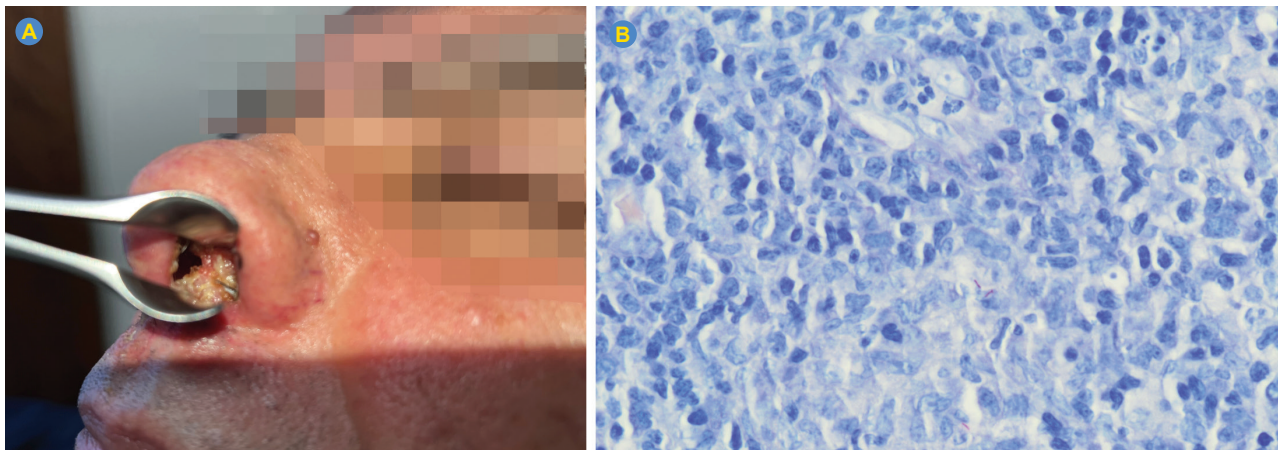

Figure 1 – (A) Anterior rhinoscopy revealing septal lesion; (B) Staining with Ziehl-Neelsen revealing acid-alcohol resistant bacilli (original magnification x600)

PROTECTION OF HUMANS AND ANIMALS

The authors declare that the procedures were followed according to the regulations established by the Clinical Research and Ethics Committee and to the Helsinki Declaration of the World Medical Association updated in 2013.

DATA CONFIDENTIALITY

The authors declare having followed the protocols in use at their working center regarding patients' data publication.

PATIENT CONSENT

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COMPETING INTERESTS

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Unveiling the First *Staphylococcus argenteus* Infection in Portugal

Descrição da Primeira Infeção por *Staphylococcus argenteus* em Portugal

Keywords: Portugal; Staphylococcal Infections; Staphylococcus argenteus
Palavras-chave: Infecções Estafilocócicas; Portugal; Staphylococcus argenteus

Dear Editor,

Staphylococcus argenteus is a novel species described in 2015, belonging to a divergent *Staphylococcus aureus* lineage.[1] Since then, the detection of *S. argenteus* infections increased worldwide, although it remains undistinguished from *S. aureus* by standard non-molecular methods.[1-4]

In December 2021, a 71-year-old man was admitted to the intensive care unit with respiratory failure associated with COVID-19 pneumonia. On day six of intubation, the tracheal aspirate was collected after the detection of purulent sputum associated with fever and increased systemic inflammatory markers.

At the microbiology laboratory, the bacteriological examination revealed a non-pigmented and greyish creamy colony with beta-haemolysis on blood agar (Fig. 1A) and a positive coagulase agglutination test. A putative *Staphylococcus aureus* was initially identified (labelled as ULSM26) through the automated identification systems VITEK®MS (bioMérieux) and susceptibility testing was performed on Vitek®2 (bioMérieux) with the AST-P648 card. Penicillin and vancomycin susceptibility were assessed by disc diffusion and the agar gradient test, respectively, according to the EUCAST breakpoints. The genetic background of ULSM26 was assessed by *spa* typing and detection of *mecA*, *pvl* and other virulence determinants were carried out by PCR.[5,6]

A methicillin-susceptible *S. aureus* (MSSA), with non-multiresistant profile, except to penicillin and tetracycline, was reported and the patient received ceftriaxone with a favourable clinical evolution.

Molecular characterization identified a *spa* type t5078, associated with clonal complex 75 and suggestive of a *Staphylococcus argenteus* species, which was confirmed by NRPS gene amplification (Fig. 1B).[1] Neither *mecA*, *pvl*, or other virulence genes were detected on USLM26, except the staphylococcal immune evasion cluster genes *sak* (staphylokinase) and *scn* (staphylococcal complement inhibitor).

Previous studies suggest that the frequency of healthcare-associated infections, morbidity and mortality are comparable to those of *S. aureus*.[2] Although resistance rates seem to be lower in *S. argenteus*, penicillin-resistant strains are common and methicillin resistance is prevalent in Europe and Australia.[3] Furthermore, a wide variety of virulence

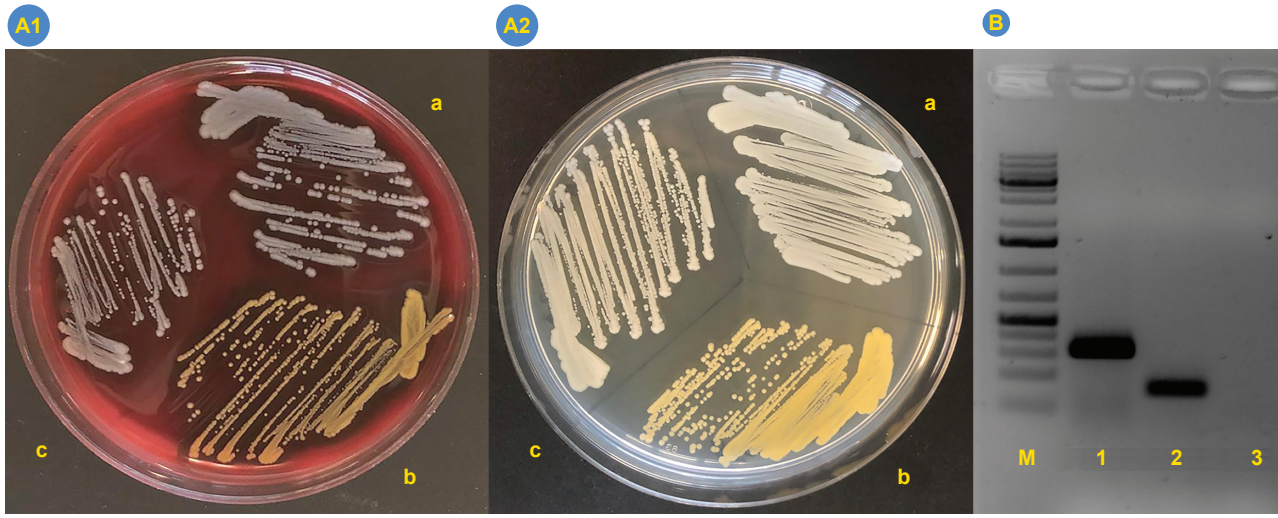

Figure 1 – Comparison between *Staphylococcus argenteus* (a) and *Staphylococcus aureus* (b and c) colonies after 24 hour incubation in non-selective media (triptic soy agar - TSA, Becton & Dickinson, Sparks, MD, USA) at 35°C with (A1) and without (A2) blood supplement. (a) *S. argenteus* ULSM26; (b) *S. aureus* ATCC6538; (c) *S. aureus* ATCC25923 (control strain). PCR amplicons of the nonribosomal peptide synthetase gene (NRPS).
1 – *S. argenteus* ULSM26 (340 bp); 2 – *S. aureus* BAA-42 (160 bp); 3 - negative control; M - 1 kb plus DNA ladder (B).

determinants have been described in *S. argenteus*, confirming its pathogenic potential.[1-3]

While *S. aureus* are endemic in Portuguese hospitals with 25% of methicillin-resistance in 2021[5], *S. argenteus* had not been previously detected. We believe that this identification rate is due to the fact that this microorganism is not present in the database routinely used in the laboratory (VITEK®MS System IVD 3.2). However, the new version of this system, which will be implemented soon, already incorporates *Staphylococcus argenteus*. In order to improve future decisions regarding surveillance, clinical relevance, and infection control, it is necessary to have updated laboratory equipment. Meanwhile, it is worth retaining the message that *S. argenteus,* an emerging pathogen with the ability to cause serious infection, is already circulating in our hospitals.

AUTHOR CONTRIBUTIONS

TC, MF: Data collection and writing of the manuscript.
VA, HL: Critical review and approval of the manuscript.

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PATIENT CONSENT

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COMPETING INTERESTS

The authors have declared that no competing interests exist.

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Three Autochthonous Cases of Strongyloidiasis: An Endemic Tropical Disease Underdiagnosed in Portugal

Três Casos Autóctones de Estrongiloidíase: Uma Doença Tropical Subdiagnosticada Em Portugal

Keywords: Portugal; Strongyloides stercoralis; Strongyloidiasis
Palavras-chave: Estrongiloidíase; Portugal; Strongyloides stercoralis

Strongyloides stercoralis is a nematode with worldwide distribution, and a frequent cause of infection in tropical and subtropical regions.[1–5] Although sporadic imported and autochthonous cases have been described in Southern Europe,[3–5] there is no recent epidemiological data from Portugal.

Infection is more common in areas with poor sanitary conditions as it frequently occurs after skin exposure to contaminated soil with larvae. In Portugal, several autochthonous cases were described until 1985, suggesting endemic *foci*, mainly in the district of Coimbra.[1,2] After 1986 (when Portugal joined what was then known as the European Economic Community), sanitary conditions improved and the infection became underdiagnosed.[2]

The parasite can complete its lifecycle within the human host with persistent autoinfection. If not correctly eradicated, the parasitosis may persist for decades, with periods of remission and recurrence of symptoms – mainly gastrointestinal, respiratory, or cutaneous. Peripheral intermittent eosinophilia is common, but its absence does not exclude the diagnosis.[1]

In immunocompromised individuals (particularly those with impaired cellular immunity),[3] hyperinfection syndrome may occur: an accelerated autoinfection cycle with disseminated strongyloidiasis, associated with a high morbimortality. Therefore, eradication is particularly important in immunosuppressed or immunosuppression candidates.[1,3]

In 2020, Pinto *et al*[1] presented one of the first autochthonous cases of strongyloidiasis in Portugal since 1985[5]: the case of a 69-year-old farmer with abdominal pain and eosinophilia.

We present three cases of strongyloidiasis diagnosed at our institution between 2017 and 2018. These individuals were born between 1935 and 1958 in Matosinhos, Vila do Conde and Amarante, with no travel to endemic areas reported before diagnosis.

One patient was completely asymptomatic, but parasite eradication was performed as the patient was a candidate for immunosuppressive therapy. In the other two situations, symptoms or signs compatible with chronic infection were described: diarrhea after the initiation of rituximab and high-dose corticosteroids, and marked eosinophilia (49%, 5800 eosinophils/μL). In the latter, eosinophilia improved dramatically after treatment.

In all cases, parasitological stool examinations were negative, and the diagnosis was confirmed by immunoenzymatic assays.

The authors wish to draw attention to the existence of chronic carriers of *S. stercoralis,* who may have contracted the infection decades earlier. This parasitic infection is presumably forgotten and underdiagnosed due to the low clinical suspicion in patients without a history of travel to endemic regions. The consequences of hyperinfection syndrome after immunosuppression are dismal, and a high degree of suspicion is needed, particularly in patients with previous or current precarious sanitary conditions.

AUTHOR CONTRIBUTIONS

SRO: Data collection and writing of the manuscript.
CB, SMS: Data collection.
IN, SJ: Critical review of the manuscript.

PROTECTION OF HUMANS AND ANIMALS

The authors declare that the procedures were followed according to the regulations established by the Clinical Research and Ethics Committee and to the Helsinki Declaration of the World Medical Association updated in 2013.

DATA CONFIDENTIALITY

The authors declare having followed the protocols in use at their working center regarding patients' data publication.

PATIENT CONSENT

Obtained.

COMPETING INTERESTS

SRO has received support for attending the National Congress of Infectious Diseases, the congress 'Infection and Sepsis' and a course on osteoarticular infections from

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Tuberculosis Screening of Ukrainian Refugees in Portugal

Rastreio de Tuberculose em Refugiados da Ucrânia em Portugal

Keywords: Mass Screening; Portugal; Refugees; Tuberculosis/epidemiology; Ukraine
Palavras-chave: Portugal; Rastreio; Refugiados; Tuberculose/epidemiologia; Ucrânia

To the Editor,

On February 24, 2022, Russia launched a military offensive in Ukraine that has already caused an undetermined number of deaths and more than 11 million refugees.[1,2] At least 48 000 refugees had applied for temporary protection in Portugal by August 2022.[1] Ukraine is one of the tuberculosis (TB) high-priority countries in the World Health Organization (WHO) European Region and one of the nine countries globally with a high burden of multidrug-resistant TB.[3]

Even though there are no specific recommendations regarding TB screening among refugees in Portugal, according to Portugal's Directorate-General of Health all citizens coming from the Ukraine should be asked about symptoms, exposure, comorbidities or risk factors for disease progression.

In Portugal, TB patients are diagnosed and treated free-of-charge, regardless of the country of origin and legal status.[4]

We conducted a cross-sectional study using an electronic survey. The aim was to understand what adjustments the different national outpatient TB centers (OTBC) made to comply with TB screening in Ukrainian refugees. This study was previously approved by the Ethics Committee of the Northern Regional Health Administration (RHA). The survey was sent five times via email to all OTBC coordinators to increase the response rate. Responses were collected during August 2022.

Twenty-nine OTBC coordinators responded to the questionnaire, from a total of 61 (response rate of 47.5%). The characteristics of the OTBC coordinators and the response rate by region are summarized in Table 1.

Twenty-three OTBC (79.3%) mentioned that Ukrainian refugees underwent TB screening approximately fourteen days after arrival in Portugal. The screening process included a symptom questionnaire and chest radiography (52.2%). Additionally, 47.8% (n = 11) reported including latent TB infection (LTBI) screening with tuberculin skin test and/or interferon gamma release assay. In 65.2% (n = 15) of the OTBC carrying out TB screening of Ukrainian refugees, more than 20 were performed.

OTBC coordinators flagged only one patient with a previous diagnosis of TB. There were 13 diagnoses of LTBI, mainly in the Northern RHA (76.9%). In this region, most of the centers (55.6%) only included a symptom questionnaire and chest radiography. Treatment for LTBI was carried out in seven patients (53.8%). No new TB diagnoses were made.

Table 1 – Outpatient Tuberculosis Centers coordinators' characteristics and response rate by Regional Health Administration

OTBC coordinators' characteristics	**n** (%)
Profession	
Family Physician	12 (41.4)
Nurse	8 (27.6)
Other medical specialty	6 (20.7)
Pulmonologist	3 (10.3)
OTBC response rate	**n/total** (%)
RHA North	18/21 (85.7)
RHA Algarve	5/9 (55.5)
RHA Lisbon and Tagus Valley	4/12 (33.3)
RHA Center	2/11(18.2)
Total	**29/61 (47.5)**

RHA: Regional Health Administration

Nonetheless, the following problems were raised: difficulties in the mobility of refugees to another city, refusal to perform chest radiography, linguistic barrier, lack of human resources, and response rate across all RHA.

Tuberculosis screening is a current challenge and ensures that people with a previous diagnosis continue to be medically treated. However, it is not surprising that the European Centre for Disease Prevention and Control and WHO Europe have recommended screening and testing only for certain refugee groups, such as people living with the human immunodeficiency virus or those who are contacts of TB patients. It is essential to balance benefits and harms, such as stigmatization, discrimination, resource use, and mental health issues.

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AUTHOR CONTRIBUTIONS

RF: Study design, data analysis and interpretation, writing of the manuscript.

RD: Study design, data interpretation, critical review of the manuscript.

MV: Data interpretation and critical review of the manuscript.

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