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Long-COVID: Um Desafio para a Comunidade Médica e para o Serviço Nacional de Saúde

Long-COVID: A Challenge for the Medical Community and the National Health Service

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Palavras-chave: COVID-19/complicações; Portugal
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Decorridos mais de 18 meses desde o diagnóstico do primeiro caso de COVID-19, esta continua a ser uma doença nova que desafia a comunidade científica a aprender em tempo real. Os relatos dos doentes recuperados, referindo sintomas e sinais que persistem semanas a meses após a resolução da infecção aguda, obrigam-nos a reflectir sobre a história natural da infecção por SARS-CoV-2. Embora não exista ainda uma definição consensual, a *long*-COVID é uma condição caracterizada por manifestações clínicas multissistémicas, do foro respiratório, cardiovascular, neurológico, gastrointestinal, renal e/ou músculo-esquelético, que surgem habitualmente na fase aguda da infecção ou imediatamente depois, não são explicáveis por um diagnóstico alternativo e persistem além das quatro semanas de doença.[1]

Os sintomas podem manifestar-se isoladamente ou em associações diversas, surgir vários meses após a doença aguda e ter uma evolução cíclica com períodos de remissão e de recidiva.

A utilização de nomenclaturas diferentes para designar esta entidade e a ausência de critérios universais definidores do diagnóstico têm comprometido a identificação de casos clínicos, a investigação da doença, nomeadamente a sua fisiopatologia, e a partilha de informação.

Não existem dados da prevalência da *long*-COVID em Portugal. Os números referentes a outros países são díspares e reflectem a ausência de uma uniformização de terminologia e de critérios de diagnóstico. No dia 31 Julho de 2021, tinham sido diagnosticados em Portugal cerca de 968 000 casos de infecção por SARS-CoV-2.[2] Se assumirmos que 10% a 30% destes doentes desenvolvem sintomas persistentes, como é referido por alguns autores, facilmente reconhecemos a sobrecarga adicional que esta doença representará para o Serviço Nacional de Saúde (SNS) e para a economia do país.[3] Serão mais de 96 000 doentes, a maioria numa faixa etária profissionalmente activa, a necessitarem de cuidados médicos a médio-longo prazo e que não conseguem retomar a actividade profissional, nos primeiros meses após a infecção, devido ao compromisso funcional causado pela doença.

Foram os doentes recuperados, mas com sintomas que comprometiam a sua qualidade de vida e desempenho profissional, que, sentindo-se incompreendidos e insatisfeitos com as soluções apontadas pelos profissionais de saúde, deram o alerta para esta doença nova e reclamaram o seu reconhecimento. Recusaram o rótulo de "dano psicológico", organizaram-se em associações e grupos de apoio e divulgaram *online* o conceito de *long*-COVID. Foram, e ainda são, usadas outras designações, como síndrome pós-COVID-19 e COVID-19 crónica, mas *long*-COVID parece ser a mais consensual por ter implícito o *continuum* de sintomas da fase aguda, sem o corte abrupto sugerido pela "síndrome pós-COVID" e o peso da cronicidade de outros termos. Mais recentemente o termo *long*-COVID foi reconhecido pela comunidade científica, surgiu em revistas científicas com revisão por pares e tem sido tema de editoriais e artigos de revisão e de opinião em diversas revistas científicas.[1,3,4]

Alguns autores referem-se à *long*-COVID como uma síndrome pós-infecciosa idêntica à encefalomielite miálgica (EM), síndrome de fadiga crónica, associada ao SARS-CoV-1, ao vírus Epstein-Barr e à *Coxiella burnetti*, e à síndrome pós-doença de Lyme. A EM é uma doença crónica multissistémica caracterizada por um conjunto de sintomas, fadiga, distúrbios do sono, alterações neurocognitivas, intolerância ortostática e mal-estar pós-esforço, agravados pelo *stress* e esforço físico. Não há actualmente evidência científica que o SARS-CoV-2 constitua um *trigger* para a EM. Apesar dos mecanismos etiopatogénicos da *long*-COVID não terem sido ainda esclarecidos, são apontadas várias hipóteses, nomeadamente a persistência do SARS-CoV-2 nos tecidos, a disfunção imunitária e a resposta inflamatória desencadeadas pelo vírus e fenómenos disautonómicos. A presença do vírus na mucosa gastrointestinal foi documentada histologicamente em um terço de doentes submetidos a biópsias quatro meses depois da infecção aguda.[4] Contudo, a presença do vírus nos tecidos não é nem condição necessária, nem sinónimo de persistência da doença. Daniel Altmann *et al*, num artigo de opinião publicado na *BMJ*, refere que os mecanismos

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fisiopatológicos subjacentes às doenças auto-imunes podem constituir um modelo conceptual relevante para o padrão de sintomas da *long*-COVID.[4] Faz alusão ao lúpus, enquanto doença de um sistema único, o sistema imunitário, mas com afecção de vários outros e manifestações multissistémicas, e à evolução clínica da esclerose múltipla, com padrão de remissão-recaída idêntico ao de alguns casos de *long*-COVID. Refere-se ainda às manifestações de auto-imunidade e resposta inflamatória persistente associadas a outras infecções virais, como são os casos do Ébola, o Chikungunya ou o Epstein-Barr. As semelhanças da *long*-COVID com outras patologias, infecciosas ou auto-imunes, são inegáveis, mas na fase inicial de uma doença nova, estabelecer analogias com outras doenças pode ser redutor e comprometer a investigação de um modelo fisiopatológico com características singulares. O conhecimento da fisiopatologia da *long*-COVID é crucial para o desenvolvimento de intervenções terapêuticas, que podem vir a modificar a trajectória da doença e mitigar o seu impacto. A persistência do vírus nos tecidos reforça a importância da investigação de fármacos anti-víricos e a disfunção imunitária associada ao SARS-CoV-2, além das implicações terapêuticas, alerta-nos para o risco futuro de patologias auto-imunes nos doentes que tiveram COVID-19.

Os estudos observacionais de *follow-up* de doentes com *long*-COVID, divulgados em revistas científicas com revisão por pares, oferecem-nos uma caracterização detalhada das manifestações clínicas e uma perspectiva da sua prevalência e evolução temporal.[1] Verificamos que os doentes diferem entre si, no que diz respeito ao padrão de sintomas e à gravidade clínica, e que a maioria se mantem sintomática 6 meses após a infecção aguda. Contudo, não sabemos quais e quantos doentes irão recuperar e os que vão apresentar sintomas incapacitantes no futuro. Quanto aos factores de risco de evolução para *long*-COVID, não foi demonstrada uma correlação directa entre a gravidade clínica da doença aguda e o risco de *long*-COVID e o pressuposto que os doentes com doença crítica têm uma maior probabilidade de desenvolver sintomas persistentes está errado. A idade, o sexo masculino e a obesidade, factores de risco para COVID-19 grave, não estão associados a risco aumentado de *long*-COVID. Deve ser investigado o potencial de risco de outros factores, demográficos e clínicos, e o impacto dos tratamentos instituídos na infecção aguda não deve ser descurado.

A *long*-COVID constitui um desafio para a comunidade médica. A novidade da doença e a ausência de recomendações de organizações e sociedades científicas, que guiem a marcha diagnóstica e tratamento, gera ansiedade e insegurança. Além da fisiopatologia, desconhecemos a epidemiologia, o curso natural da doença e as potenciais sequelas. Estamos, em muitos aspectos, perante o desconhecido, mas a gravidade e dimensão do problema exige uma resposta sólida e estruturada. Os médicos que acompanham estes doentes têm de investir na colheita, registo e organização de dados, promover a criação de plataformas nacionais de registo e organizar-se em grupos de trabalho que facilitem a troca de informações. Mas, citando Daniel Altmann, é necessário ir além dos estudos observacionais e promover estudos de intervenção. A inclusão de doentes com *long*-COVID em ensaios clínicos é já uma prioridade reconhecida por alguns países e centros de investigação. Destaco o trabalho da Organização Mundial de Saúde (OMS) e o programa SARS-CoV-2 *Recovery Cohort*, uma iniciativa do National Institutes of Health, que inclui estudos prospectivos de coorte que pretendem avaliar os efeitos da infecção a longo prazo e acompanhar a trajectória de sintomas ao longo do tempo.[5]

Steven Philips *et al*, num artigo recentemente publicado na *NEJM*, refere-se à *long*-COVID como o próximo desastre nacional de saúde.[3] Como podemos evitar que uma condição quase desconhecida, que afecta um número tão significativo de doentes, abale um SNS já com tantas fragilidades? Através de uma resposta coordenada da política nacional de saúde, investindo na prevenção da infecção e na investigação da doença, definindo planos de intervenção adaptados à realidade do país e ajustáveis à evolução da pandemia e, acima de tudo, gerindo, de forma realista e responsável, os recursos humanos e as instituições.

A *long*-COVID tem características singulares. O alerta para os sintomas persistentes foi dado pelos doentes, os mesmos que reclamaram o seu reconhecimento como "doença nova", que a mediatizaram e alertaram para o impacto que poderia ter na saúde, na economia e na sociedade. A comunidade médica mostrou-se ambivalente e reconheceu, ao olhar para o passado, a possibilidade de estar perante uma nova síndrome pós-viral, mas não afastou, de imediato, o contributo de uma componente psicológica. A abordagem desta doença é também um exercício de humildade. Não há sinais ou sintomas patognomónicos, não há biomarcadores da doença nem testes diagnósticos que a afirmem ou excluam. O que podemos fazer? Tão simples quanto assumir que não sabemos, identificar o que precisamos saber e melhorar e criar as condições para que isso aconteça.

CONFLITOS DE INTERESSE

A autora declara não ter conflitos de interesse relacionados com o presente trabalho.

REFERÊNCIAS

1. Crook H, Raza S, Nowell J, Young M, Edison P. Long covid – mechanisms, risk factors, and management. BMJ. 2021;374:n1648.
2. Portugal. Worldmeter. [acedido 2021 Jul 31]. Disponível em; https://www.worldometers.info/coronavirus/country/portugal/
3. Philips S, Williams M. Confronting our next national health disaster – Long-Haul Covid. N Engl J Med. 2021. DOI: 10.1056/NEJMp2109285.
4. Altmann D, Boyton R. Decoding the unknowns in long covid. BMJ. 2021;372:n132.
5. National Institutes of Health. [acedido 2021 Jul 31]. Disponível em: covid19.nih.gov/sites/default/files/2021-02/PASC-ROA-OTA-Recovery-Cohort Studies.pdf.

Physical Activity During the COVID-19 Pandemic in People with Severe Mental Disorders: An Overview of the Portuguese Reality

Atividade Física Durante a Pandemia de COVID-19 em Pessoas com Doença Mental Grave: Perspetiva Sobre a Realidade Portuguesa

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Coronavirus disease 2019 (COVID-19) is a public health emergency of international concern that has led to a worldwide crisis with dramatic consequences for life, health, economy, and society.[1] In Portugal, like in many other countries around the world, the strategies to manage the pandemic have mostly revolved around imposing restrictions. In particular, the social distancing measures and lockdown requirements resulted, among others, in the temporary closing of many crowded environments including fitness centres, public gyms and sports facilities. Albeit essential to decrease the spread of the virus, these measures reduced the number of opportunities to remain physically active, and probably led to a decrease in the physical activity (Physical Activity is defined as any bodily movement produced by skeletal muscles that results in energy expenditure. Physical activity in daily life can be categorized into occupational, sports, conditioning, household, or other activities) levels, while the amount of time spent in sedentary behaviours (e.g., time spending seated or laid down, and watching TV) potentially increased, as described elsewhere.[2,3] Moreover, the COVID-19 pandemic has posed major challenges for maintaining mental health. For instance, both individuals with and without mental disorders may experience negative psychological effects like distress, and symptoms of depression and anxiety.[1,4] Although the impact of COVID-19 on the physical activity in those without pre-existing psychiatric disorders has been covered extensively, there has been a limited focus on individuals with severe mental disorders (such as bipolar disorder, schizophrenia, and other psychosis) and emerging psychopathology. This is particularly disconcerting because individuals with severe mental disorders already tend to have low levels of physical activity and present higher risk of infection and of suffering complications from COVID-19 compared to those without a psychiatric disorder, due to the disorder per se, comorbid conditions (obesity, diabetes, hypertension) and cognitive deficits.[2,5] Moreover, with lockdown restrictions, individuals with severe mental disorders can be more susceptible to stress, and consequently, relapses or worsening of the disease course can occur.[2,6,7] Sole *et al*[2] evaluated the effects of the COVID-19 pandemic and lockdown measures between community controls and patients with mental disorder during the state of emergency and found that patients reported engaging in less physical activity compared with the community controls. Additionally, the study also revealed that during the lockdown, symptoms of anxiety and depression, as well as weight gain, sleep changes and tobacco consumption were more prevalent in the mental disorder group compared to community controls. Nonetheless, this vulnerable population relies on psychiatric services, such as institutions, programs, or group support to encourage and promote a healthy lifestyle including physical active behaviour.[8] Unfortunately, due to the burden imposed by the pandemic, namely during the period of the state of emergency, the Portuguese National Health System had to adjust in order to ensure the response to essential care needs and the Portuguese Psychiatric and Mental Health units were included in this adjustment – limiting appointments and home visits to the most urgent cases; non-urgent outpatient appointments were carried out by telephone.[9] Moreover, the temporary closure of several ambulatory facilities for psychiatric patients, including acute day hospitals, led to an abrupt interruption in the provision of ambulatory mental health care to many patients.[9]

In this scenario, it is crucial that government, psychiatric services, and healthcare providers understand the reality of

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people with severe mental disorders in order to support their needs with adequate policies, such as ensuring the continuity of necessary community-based social and healthcare services and providing access to important information. A recent study[10] demonstrated that meeting physical activity guidelines was strongly associated with a reduced risk for severe COVID-19 outcomes among infected adults. Therefore, the promotion of physical activity should be prioritised by public health agencies and incorporated into routine medical care.[10] Due to the vulnerability of people with severe mental disorders[5] it is crucial to improve their physical activity levels by implementing innovative and effective strategies.

Firstly, it is crucial to understand that people with severe mental disorders often exhibit limited motivation to engage in physical activity and to maintain an active lifestyle.[11-13] Several factors can explain this behaviour, namely, the presence of negative and depressive symptoms, low self-efficacy and lack of confidence and somatic co-morbidities associated with the disorder (e.g., metabolic syndrome and clinical pain).[11-13] Therefore, the implementation of motivational strategies is essential to engage this population in physical activity. Strategies such as a relationship of trust with mental healthcare professionals, positive interactions with peers, and engaging in physical activity in a non-clinical environment are linked with starting and maintaining physically active behaviours.[12,13] However, due to the COVID-19 pandemic, all these motivational processes became difficult to enforce. Therefore, an adjustment to the conventional approach became necessary. For example, during telephone appointments, online psychoeducation programs or online mindfulness-based interventions, mental healthcare professionals supported by qualified exercise professionals, could spend a few minutes to discuss the details of a healthy lifestyle and encourage patients to perform more physical activity. Some strategies can be suggested as discussed below.

Increasing physical activity levels

Costa *et al*[11], in a Portuguese sample of patients with schizophrenia, demonstrated that autonomous motivation (i.e., when patients behave with a full sense of volition and choice) is a significant predictor of physical activity. The authors suggested that clinical practice guidelines should highlight the importance of autonomous motivation to improve attitudes towards physical activity. Evidence revealed that in order to increase the level of autonomous motivation, clinicians should minimize pressure, adopt supportive language, and promote pleasant activities in a positive environment.[12,13] For example, qualified exercise professionals with expertise in exercise (Exercise is a subset of physical activity that is planned, structured, and repetitive and has as a final or an intermediate objective the improvement or maintenance of physical fitness) prescription, and additional knowledge regarding psychopathology, can help patients create a new weekly schedule that should be both enjoyable and feasible. Due to the pandemic, the regular daily routines have changed, including the routine to engage in physical activity. Therefore, a new and personalized schedule is important to provide some structure to individuals with severe mental disorders. Qualified exercise professionals can provide support and guidance to create the schedule, for instance, by suggesting when to include the exercise sessions, the duration of each session, what type of activity (e.g., walking, yoga, circuit training), clarify what will be the necessary equipment (if applicable), and the intensity (i.e., low, moderate, and high). It is important to consider the physical activity recommendations from the World Health Organization[14]: adults should do at least 150–300 minutes of moderate-intensity aerobic physical activity; or at least 75–150 minutes of vigorous-intensity aerobic physical activity throughout the week. Combinations of moderate and vigorous intensity exercise can be performed in order to meet this recommendation. Adults should also do muscle-strengthening activities at moderate intensity or greater that involve all major muscle groups on two or more days a week. In order to help achieve these recommendations, workout resources (e.g., exercise illustrations or exercise videos) should be provided. These can be facilitated by online services or, if this is not possible, copies or flyers with exercise illustrations (e.g., *Manual de Boas Práticas em Atividade Física na Doença Mental*)[15] can be sent by mail.

Decreasing sedentary behaviour

A systematic review and meta-analysis[16] analysed sedentary time of people with psychosis and the authors found that this population spent more than 11 hours of their waking day being sedentary. These high levels of sedentary behaviour, along with the decline of cardiovascular health in this population, highlights the need for approaches specifically targeting sedentary behaviour.[16] The World Health Organization[14] recommends that adults should limit the amount of time spent being sedentary. In order to reduce sedentary time, it is important to avoid long periods of inactivity – for example by using external cues such as advert breaks or set alarms to perform a movement break (e.g., stretching, five jumps, walking to the door and returning, climbing stairs) when watching TV. In the same way, performing daily activities like cleaning and cooking, gardening, and farming; performing a short exercise workout (e.g., relaxation exercises, abs, stretching, strength condition using body weight and/ or objects in the house [e.g., chairs, water bottles, rice package]) are also good strategies to reduce sedentary time and consequently improve physical activity

levels. Walking is also a good activity to increase physical activity levels (but not enough to meet the above physical activity recommendations), for example people with severe mental disorder should be encouraged to choose walking to the nearest supermarket instead of using a car or public transport.

Furthermore, the use of devices and social support could also be good strategies to maintain physical activity levels and decrease sedentary behaviour in people with severe mental disorders. There is evidence demonstrating that mental health technologies for activity tracking are feasible and acceptable for use among patients with severe mental disorders.[17] Apps and devices – for example activity monitors, set with reminders to move, can track activity levels and step counts. Mental healthcare professionals can motivate each person to increase their physical activity level every day, for example by increasing the number of steps or reduce the time spent in sedentary behaviours. It is important to take into consideration that this vulnerable population can present significant difficulties in terms of access to technologies or resources that are commonly used by the general population. Regarding social support, a recent systematic review[18] showed that family members play a pivotal role in supporting health parameters, like cardiovascular care of people with severe mental disorders. Therefore, mental healthcare professionals can encourage the household to adopt a healthy lifestyle as well – sharing periods of physical activity with others in the household can be more motivating to people with severe mental disorders. Finally, social support will be beneficial for both physical and mental health.

These strategies, along with a prompt and effective response in the other domains of the patient's life (e.g., treatment engagement, family support, social inclusion), holds the potential to support people with mental disorders to maintain physical active behaviours throughout the pandemic, promoting physical and mental well-being and preventing the widening of health inequalities.[19]

AUTHORS CONTRIBUTION

RC: Drafted the manuscript.

TB, EG: Provided input and critical revision.

RC: Reviewed the manuscript.

COMPETING INTERESTS

The authors declare they have no conflicts of interest.

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REFERENCES

1. Holmes EA, O'Connor RC, Perry VH, Tracey I, Wessely S, Arseneault L, et al. Multidisciplinary research priorities for the COVID-19 pandemic: a call for action for mental health science. Lancet Psychiatry. 2020;7:547-60.
2. Sole B, Verdolini N, Amoretti S, Montejo L, Rosa AR, Hogg B, et al. Effects of the COVID-19 pandemic and lockdown in Spain: comparison between community controls and patients with a psychiatric disorder. Preliminary results from the BRIS-MHC STUDY. J Affect Disord. 2021;281:13-23.
3. Stockwell S, Trott M, Tully M, Shin J, Barnett Y, Butler L, et al. Changes in physical activity and sedentary behaviours from before to during the COVID-19 pandemic lockdown: a systematic review. BMJ Open Sport Exerc. 2021;7:1-7.
4. Kontoangelos K, Economou M, Papageorgiou C. Mental Health Effects of COVID-19 Pandemia: A Review of Clinical and Psychological Traits. Psychiatry Investig. 2020;17:491-505.
5. Yao H, Chen JH, Xu YF. Patients with mental health disorders in the COVID-19 epidemic. Lancet Psychiatry. 2020;7:e21.
6. Kozloff N, Mulsant BH, Stergiopoulos V, Voineskos AN. The COVID-19 Global Pandemic: Implications for People With Schizophrenia and Related Disorders. Schizophr Bull. 2020;46:752-7.
7. Yocum AK, Zhai Y, McInnis MG, Han P. Covid-19 pandemic and lockdown impacts: A description in a longitudinal study of bipolar disorder. J Affect Disord. 2021;282:1226-33.
8. Muruganandam P, Neelamegam S, Menon V, Alexander J, Chaturvedi SK. COVID-19 and Severe Mental Illness: Impact on patients and its relation with their awareness about COVID-19. Psychiatry Res. 2020;291:113265.
9. Goncalves-Pinho M, Mota P, Ribeiro J, Macedo S, Freitas A. The Impact of COVID-19 Pandemic on Psychiatric Emergency Department Visits - A Descriptive Study. Psychiatr Q. 2020:1-11.
10. Sallis R, Young DR, Tartof SY, Sallis JF, Sall J, Li Q, et al. Physical inactivity is associated with a higher risk for severe COVID-19 outcomes: a study in 48 440 adult patients. Br J Sports Med. 2021(in press). doi: 10.1136/bjsports-2021-104080.
11. Costa R, Bastos T, Probst M, Seabra A, Vilhena E, Corredeira R. Autonomous motivation and quality of life as predictors of physical activity in patients with schizophrenia. Int J Psychiatry Clin Pract. 2018;22:184-90.
12. Farholm A, Sorensen M. Motivation for physical activity and exercise in severe mental illness: A systematic review of intervention studies. Int J Ment Health Nurs. 2016;25:194-205.
13. Vancampfort D, De Hert M, Stubbs B, Ward PB, Rosenbaum S, Soundy A, et al. Negative symptoms are associated with lower autonomous motivation towards physical activity in people with schizophrenia. Compr Psychiatry. 2015;56:128-32.
14. World Health Organization. WHO guidelines on physical activity and sedentary behaviour. Geneva: WHO; 2020.
15. Bastos T, Costa R, Gomes E, Pizarro A, Corredeira R. Manual de Boas Práticas em Atividade Física na Doença Mental. Porto: Faculdade de Desporto da Universidade do Porto; 2019.
16. Stubbs B, Williams J, Gaughran F, Craig T. How sedentary are people with psychosis? A systematic review and meta-analysis. Schizophr Res. 2016;171:103-9.
17. Naslund JA, Aschbrenner KA, Barre LK, Bartels SJ. Feasibility of Popular m-Health Technologies for Activity Tracking Among Individuals with Serious Mental Illness. Telemed E-Health. 2015;21:213-6.
18. Zabeen S, Phua D, Mohammadi L, Lawn S. Family involvement to support cardiovascular self-management care for people with severe mental illness: a systematic review. J. Ment. Health. 2020 (in press). doi: 10.1080/09638237.2020.1818194.
19. Campion J, Javed A, Sartorius N, Marmot M. Addressing the public mental health challenge of COVID-19. Lancet Psychiatry. 2020;7:657-9.

Epidemiological Profile in Portugal of Breast Implant-Associated Anaplastic Large Cell Lymphoma: A Cross--Sectional Study

Perfil Epidemiológico em Portugal do Linfoma Anaplásico de Grandes Células Associado a Implantes Mamários: Um Estudo Transversal

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ABSTRACT

Introduction: Breast implant-associated anaplastic large cell lymphoma (BIA-ALCL) is a rare T-cell neoplasm that is predominantly associated with the use of textured implants. Recently, several countries have tried to clarify their epidemiological profile of BIA-ALCL. This study aims to estimate the number of cases of BIA-ALCL in Portugal and to describe the pattern of use of breast implants at a national level.

Material and Methods: This is a cross-sectional study including 57 healthcare institutions - 29 public hospitals and 28 private institutions. Each department of Plastic, Reconstructive and Aesthetic Surgery was asked to provide information concerning the main manufacturer(s) and respective device texture of the breast implants used, and to report the number of registered cases of BIA-ALCL.

Results: In our study sample, the response rate was 58%. In our sample, most hospitals reported using textured breast implants from Mentor (45.45%), Allergan (42.42%) and Polytech (39.39%). Only one private institution referred using smooth-coated implants from Mentor and Motiva. Despite several hospitals reporting late-onset seromas, there was only one confirmed case of BIA-ALCL after proper investigation with immunohistochemistry and histological procedures.

Discussion: BIA-ALCL may represent a shift for surgeons regarding selection of implant type. Smooth-coated implants or autologous tissue represent adequate alternatives that could surpass the risks associated with textured devices.

Conclusion: In the future, the creation of a national patient registry and proper recognition of BIA-ALCL by plastic surgeons could be useful tools to clarify the impact of the disease nationally and to mitigate potential risk factors.

Keywords: Breast Implants/adverse effects; Lymphoma, Large-Cell, Anaplastic/epidemiology; Lymphoma, Large-Cell, Anaplastic/etiology; Portugal

RESUMO

Introdução: O linfoma anaplásico de grandes células associado a implantes mamários (BIA-ALCL) é uma neoplasia rara de células T predominantemente associada ao uso de próteses texturizadas. Recentemente, vários países procuraram clarificar o seu perfil epidemiológico. Este estudo pretende estimar o número de casos de BIA-ALCL em Portugal e descrever o padrão de utilização de próteses mamárias a nível nacional.

Material e Métodos: Este é um estudo transversal realizado em 57 serviços de saúde - 29 hospitais públicos e 28 instituições privadas. A cada departamento de Cirurgia Plástica, Reconstrutiva e Estética foi solicitada informação sobre os principais fabricantes e respetiva textura dos implantes mamários utilizados, bem como número de casos registados de BIA-ALCL.

Resultados: Na nossa amostra, a taxa de resposta foi 58%. Considerando o universo de respostas obtidas, a maioria dos hospitais referiu usar implantes mamários texturizados da Mentor (45,45%), Allergan (42,42%) e Polytech (39,39%). Apenas uma instituição privada mencionou utilizar implantes lisos da Mentor e Motiva. Vários hospitais reportaram a ocorrência de seromas tardios. Contudo, apenas um caso de BIA-ALCL se veio a confirmar após investigação imunohistoquímica e histológica adequada.

Discussão: O BIA-ALCL poderá determinar uma alteração do paradigma de seleção do tipo de implante mamário, onde alternativas como os implantes lisos e tecido autólogo poderão superar os riscos inerentes aos dispositivos texturizados.

Conclusão: De futuro, a criação de um registo nacional de doentes e reconhecimento do BIA-ALCL pelos cirurgiões plásticos poderão ser importantes ferramentas para clarificar o seu impacto no território nacional e mitigar potenciais fatores de risco.

Palavras-chave: Implantes Mamários/efeitos adversos; Linfoma Anaplásico Cutâneo Primário de Células Grandes/epidemiologia; Linfoma Anaplásico Cutâneo Primário de Células Grandes/etiologia; Portugal

INTRODUCTION

Recent estimates report that approximately 10 million women worldwide have breast implants[1] and that the use of these medical devices tends to rise each year, both in the cosmetic and reconstructive fields.[2] Nevertheless, several concerns have been raised regarding their safety. In 1997, the first case that suggested a possible association between breast implants and a rare T-cell neoplasm - anaplastic large cell lymphoma emerged.[3] Since then, the number of cases increased substantially and, in 2016, the World Health Organization recognized breast implant-associated

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anaplastic large cell lymphoma (BIA-ALCL) as a novel and particular type of lymphoma.[1] Currently, 626 cases of BIA-ALCL[4] and 24 deaths have been reported around the world.[5]

BIA-ALCL is a rare T-cell neoplasm that arises in the fluid or capsule surrounding the implant. All reported cases of BIA-ALCL are CD30-positive and ALK-negative, which reinforces its unique antigenic profile.[6] Most patients present with a delayed seroma while less common manifestations include a mass, regional lymphadenopathy, skin lesions or B symptoms (such as weight loss, fever and night sweats).[7] In the presence of these symptoms, patients should undergo an ultrasound, which is recommended as the first-line imaging test.[3] Ultrasound is considered to have similar or better sensitivity and specificity in the detection of an effusion or a mass compared to magnetic resonance imaging and computerized tomography.[8] The establishment of the diagnosis requires CD30 positive staining in immunohistochemistry, cytological examination showing large anaplastic cells and flow cytometry showing clonal expansion.[7] Complete capsulectomy and implant removal is the standard of treatment for localized disease,[9] whereas chemotherapy and/or antibody-drug conjugates (such as brentuximab vedotin) are recommended in more advanced stages.[10] In most cases, BIA-ALCL has an indolent course with excellent prognosis, although fatalities have also been reported.

The device texture seems to have a role in the pathogenesis of the disease. Scientific evidence suggests that BIA-ALCL is predominantly associated with the use of textured rather than smooth-coated implants[1] and the risk seems to be higher for more robustly textured or polyurethane-covered implants.[4] As of July 2019, and considering both US and global BIA-ALCL cases reported to the US Food and Drug Administration (FDA), 67% were associated with textured implants, 5% were related to smooth-coated implants and in 28% of the cases the texture was not specified.[11] Nevertheless, the FDA states that in the cases associated with smooth implants, there was prior exposure to a textured implant or a history of prior implants was unknown.[11] Manufacturers estimate that 70% to 80% of implants sold in Europe are textured, while 70% to 80% of those sold in North America are smooth.[12] This preference towards textured implants in Europe might be explained by their supposed lower rates of capsular contracture and implant rotation in comparison with smooth breast implants.[1] Furthermore, patients who require reconstructive surgery often look for a natural shape and projection of the breast, which led to the expansion of anatomic implants.[13] These devices are always textured in order to improve adherence to the surrounding capsule and prevent device rotation.[6] Moreover, tissue expanders used in two-stage breast reconstruction have textured surfaces.[6] Prolonged time of exposure to the implant seems to be a common denominator in cases of BIA-ALCL, which occurs on average nine years after implantation.[14]

Several theories have been proposed to explain the etiology of BIA-ALCL. Some authors believe that the release of silicone degradation products is capable of activating a local immune response via T helper 1/ T helper 17 cells.[15] However, the most accepted hypothesis is that textured implants, with their greater surface areas and enhanced bacterial adhesion, lead to higher rates of biofilm formation and subsequent lymphocytic activation.[9] Hu *et al* documented a significantly greater proportion of *Ralstonia* spp., a gram-negative bacteria, in BIA-ALCL specimens compared with non-tumor capsule specimens.[16] Besides, Collett *et al* reported that high-textured high-surface area implants (grade four surface) have greater potential to harbour microorganisms and, thus, carry the highest risk of BIA-ALCL.[3]

Since breast-implants are foreign bodies, concerns have been raised regarding the possibility of their implication in 'foreign-body carcinogenesis' or as an immune trigger in the ontogenesis of other cancers, such as sarcomas, hematopoietic malignancies, cervical, vulvar and lung cancers.[17] Nevertheless, a systematic review found no evidence that breast implants alter the risk of non-breast malignancies.[17] On the other hand, several implantable devices share similarities with breast prosthesis, raising questions regarding the possibility of triggering the same neoplastic response.[18] However, there was only one confirmed case of anaplastic large cell lymphoma (ALCL) in association with an orthopedic device and most implantable devices seem to be predominantly associated with B-cell lymphomas rather than with ALCL.[18]

Due to this recent scientific information, health authorities from several countries took measures to minimize the risk of BIA-ALCL. In April 2019, the French medicines agency (ANSM) suspended the distribution and demanded the withdrawal of numerous macrotextured shell and polyurethane breast implants.[19] Several manufacturers were affected by this decision, including Allergan, Polytech, and Eurosilicone. Furthermore, ANSM is presently advising surgeons to preferentially use smooth surface implants in the cosmetic and reconstructive fields. In May 2019, Canada also suspended the licenses of macrotextured breast implants.[20]

The estimation of prevalence and incidence of BIA-ALCL faces many difficulties both in the determination of the number of women with implants and the number of cases of BIA-ALCL. Poor registries, underreporting, fear of litigation, lack of awareness and cosmetic tourism are some of the obstacles that prevent a reliable assessment.[3] The highest reported incidence is in Australia and New Zealand (1/2832), whereas the lowest relative incidence is in the Eurozone, China and Brazil.[3] Until recently, Scandinavian countries had no reported cases.[3] Portugal is one of the European countries where the number of cases of BIA-ALCL remains unknown.[1]

The present study aims to estimate the number of cases of BIA-ALCL in Portugal and to describe the main brand manufacturers and texture of breast implants used at a national level.

MATERIAL AND METHODS

This is a national multicenter cross-sectional study.

Fifty-seven healthcare organizations were included in our study, of which 29 were public hospitals and 28 were private institutions. All public institutions with a department of Plastic, Reconstructive and Aesthetic Surgery in Portugal were included. We included private institutions in order to accurately represent cosmetic surgery, an important sub-field of Plastic Surgery and one of the most significant applications of breast implants. The institutions were contacted via institutional e-mail addresses and/or telephone numbers requesting a response from the Plastic Surgery Department. When available, the contact was established directly with the department. Each department was asked to provide information concerning the main manufacturer(s) and the respective device texture of the breast implants currently used at the institution (Fig. 1). Values were reported with 95% confidence intervals. For specific types of brand and texture of breast prosthesis, namely Mentor Smooth, Silimed Polyurethane, Silimed Textured, and Motiva Smooth, the confidence interval was not calculated since the number of cases was too small to ensure reliable intervals.

Additionally, we requested each department to report the number of registered cases of BIA-ALCL (Fig.1). We included all confirmed cases of BIA-ALCL ever registered in each healthcare institution after adequate diagnostic approach (CD30 positive staining in immunohistochemistry, cytological examination showing large anaplastic cells and flow cytometry showing clonal expansion), both in the context of reconstructive and aesthetic procedures. There were no restrictions regarding the sex or age of the patient.

Late-onset seromas that did not match diagnostic criteria were not considered. Incomplete responses from the departments with missing data were not included and were classified as 'non-responses'.

If a case was confirmed, surgeons were requested to provide relevant clinical data, namely the age of the patient, brand and texture of the breast prosthesis involved, number of years after implantation, treatment approach and the current status of the patient. Surveys were implemented between the 3rd March 2019 and the 14thJanuary 2020. All answers received in this period were included in the statistical analysis. The present study was approved by the Ethics Committee of Centro Hospitalar de São João.

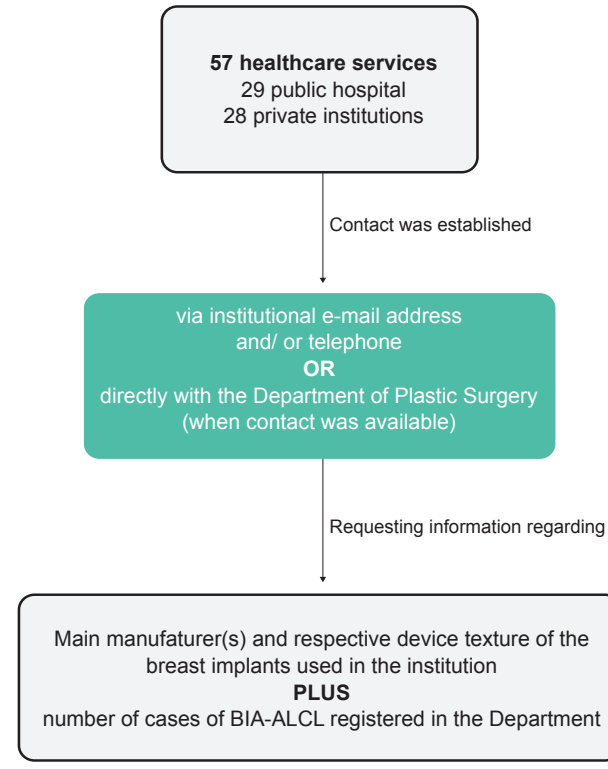

Figure 1 – Methods

RESULTS

In our universe of 57 hospitals, we obtained a total of 33 responses, which corresponds to a response rate of 58%. Considering our universe of responses, we observed that most hospitals reported the use of textured breast implants from Mentor [45.45% (29.84 - 62.02)], Allergan [42.42% (27.22 - 59.21)] and Polytech [39.39% (24.65-56.35)] (Fig.

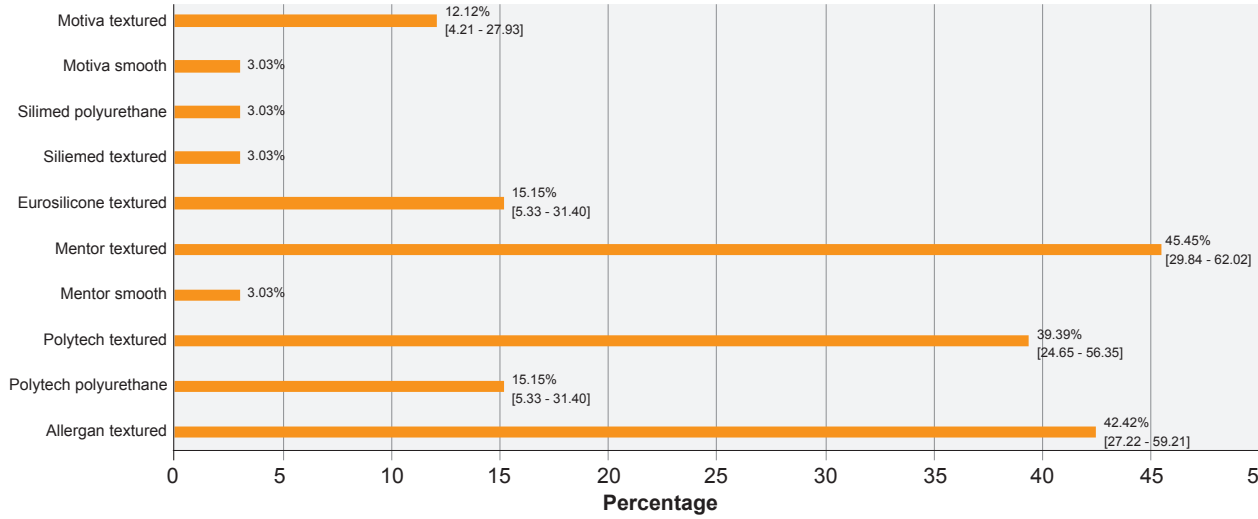

Figure 2 – Pattern of use of breast implants in the sample by texture and manufacturer

2). Polyurethane implants from Polytech and textured implants from Eurosilicone were reported to be used in a significant number of the hospitals that responded, each representing [15.15% (5.33 - 31.40)] (Fig. 2). Only one private institution reported using smooth-coated implants from Mentor and Motiva. Recently, in light of emerging scientific information, Centro Hospitalar de São João replaced the use of textured implants from Allergan with smooth implants from the same brand manufacturer.

Despite several hospitals reported having suspicious cases, there was only one confirmed case of BIA-ALCL after investigation with adequate immunohistochemistry and histological procedures (Table 1). Instituto Português de Oncologia (IPO) Porto, Centro Hospitalar de São João, Centro Hospitalar de Lisboa Central, and several others admitted having late-onset seromas that did not match the criteria to be classified as a BIA-ALCL after a proper diagnostic approach.

Table 1 – Healthcare institutions contacted (n = 57) and number of reported cases of BIA-ALCL

Districts	Hospitals/ Healthcare Centers contacted	Answers	BIA-ALCL cases
Viana do Castelo	Unidade Local de Saúde do Alto Minho	Yes	0
	Clínica Uniplástica – V. N. Famalicão	Yes	0
	Clínica Art Corpus Braga	No	-
	Clínica Santa Tecla	No	-
Braga	Hospital da Senhora da Oliveira -Guimarães	Yes	0
	Casa de Saúde de São Lázaro	Yes	0
	Misericórdia de Vila Verde	Yes	0
	Hospital de Braga	Yes	0
Vila Real	Centro Hospitalar de Trás-os-Montes e Alto Douro	Yes	0
	Centro Hospitalar de São João	Yes	0
	Hospital da Prelada	No	-
	Centro Hospitalar V.N. Gaia/Espinho	Yes	0
	CUF Porto	Yes	0
	Hospital da Luz Porto	Yes	0
	Hospital Lusíadas Porto	No	-
	Trofa Saúde	Yes	0
	Hospital da Luz Póvoa de Varzim	No	-
	Clínica Artlaser Porto	Yes	0
	CCE Porto	Yes	0
Porto	IPO Porto	Yes	0
	Hospital da Lapa	Yes	0
	Hospital da Ordem da Trindade	No	-
	Hospital Stª Maria	No	-
	Clínica Luso Espanhola	No	-
	Misericórdia de Vila Conde	Yes	0
	Misericórdia de Lousada	Yes	0
	Hospital-Escola da Universidade Fernando Pessoa	Yes	0
	Centro Hospitalar Universitário do Porto	Yes	0
	Centro Hospitalar Tâmega e Sousa	Yes	0
	Unidade Local de Saúde de Matosinhos	Yes	0
Aveiro	Centro Hospitalar Entre Douro e Vouga	Yes	0
Viseu	Centro Hospitalar Tondela-Viseu	Yes	0
	CUF Viseu	Yes	0
Coimbra	Centro Hospitalar e Universitário de Coimbra	No	-
	CUF Coimbra	No	-
	Centro Cirúrgico de Coimbra	No	-
	IPO Coimbra	No	-
	Hospital da Luz Coimbra	No	-

Table continues next page

Districts	Hospitals/ Healthcare Centers contacted	Answers	BIA-ALCL cases
Lisboa	Centro Hospitalar Lisboa Central	Yes	0
	Centro Hospitalar Universitário Lisboa Norte	Yes	0
	CUF Descobertas	Yes	0
	Clínica Faccia	No	-
	Clínica Milénio	No	-
	Clínica Europa Lisboa	No	-
	IPO Lisboa	Yes	0
	Fundação Champalimaud	Yes	1
	Hospital Beatriz Ângelo- Loures	No	-
	Centro Hospitalar Lisboa Ocidental	No	-
	Hospital Prof. Dr. Fernando Fonseca	No	-
	Hospital Garcia de Horta	No	-
Setúbal	Centro Hospitalar Barreiro Montijo	No	-
	Centro Hospitalar de Setúbal	No	-
Santarém	Hospital Distrital de Santarém	Yes	0
Évora	Hospital do Espírito Santo de Évora	No	-
Faro	Hospital de Faro	No	-
Madeira	Hospital Central do Funchal	Yes	0
Açores	Hospital do Divino Espírito Santo	Yes	0
TOTAL		33	1

The only confirmed case of BIA-ALCL in our sample was described by Fundação Champalimaud. The patient had personal history of right breast carcinoma and high genetic risk. Both breasts were intervened as part of the treatment of the oncological disease and as a prophylaxis strategy considering the genetic background. The postmastectomy reconstruction involved the use of textured implants from Allergan. Since the oncological surgery, the patient presented with three late-onset seromas, but the cytological confirmation was only established at the third recurrence. In the most recent recurrence, the patient presented with a bilateral seroma eight years after implantation which had a positive CD30 and negative ALK staining in immunohistochemistry and matched other required criteria to be classified as a BIA-ALCL. Since the tumor was bilateral and confined to the capsule, the therapeutic approach consisted of total capsulectomy and implant removal in both breasts, with subsequent surveillance.

DISCUSSION

This study described the national pattern of use of breast implants and the number of cases of BIA-ALCL in Portugal. Despite its clear association with BIA-ALCL, most healthcare institutions included in our sample reported the use of textured breast implants. Only one private institution reported using smooth-coated implants. In recent years, the popularity of textured implants among plastic surgeons increased substantially due to their allegedly lower rates of capsular contracture and implant rotation. Nevertheless, the theoretical benefit of textured devices has been increasingly questioned. Contrasting with smooth implants, textured implants have been associated with late seromas, double capsules and more recently BIA-ALCL.[21] The rate of capsular contracture between smooth and textured devices remains controversial and the superiority of textured implants regarding this key point remains yet to be proven. Even though Namnoum *et al* observed lower capsular contracture rates with textured implants,[21] two meta-analyses from 2006 found no evidence of a reduction in capsular contracture rates using textured implants when they are placed subpectorally.[5] On the other hand, a study conducted by Sieber *et al* revealed that textured implants rotate in their pockets in 42% of cases,[5] which can compromise their supposed theoretical advantage in adherence. Thus, in light of the current scenario, the benefits of smooth implants might surpass the risks of textured devices.

In our study, we identified one confirmed case of BIA-ALCL in Portugal. Despite its rarity, BIA-ALCL has a tremendous impact not only as a symbol of the medical iatrogenic potential but also in healthcare policies and management. Therefore, the number of cases reported and attempts to estimate the prevalence and incidence of this disease is rising each year. Germany and Denmark have seven reported cases, whereas Poland identified three cases at a national level.[1] Wilkinson *et al* identified 55 cases of BIA-ALCL in Australia and New Zealand between 2007 and 2016.[22] The first report of the PROFILE (Patient Registry and Outcomes for Breast Implants and Anaplastic Large Cell Lymphoma Etiology and Epidemiology) database recorded 186 distinct cases of BIA-ALCL in the USA between 2012 and 2018.[23] The estimated rates of BIA-ALCL (per implant placed) vary widely across different European regions, from 1/25 000 in

Ireland or 1/20 000 in Austria to 1/8928 in France, 1/4500 in Switzerland or even 1/2400 in the United Kingdom.[1] However, the accuracy of these estimates is questionable and is widely limited by poor registries, underreporting, fear of litigation and cosmetic tourism.[3]

While Australia and New Zealand have the highest reported incidence rate, BIA-ALCL appears to be an extremely rare event in Asians, Africans and Native American descendants.[3] These marked variations suggest that ethnicity and genetic factors may also be involved in the pathogenesis of the disease. Mutations in Janus kinase and STAT3 have been described in relation to BIA-ALCL cases, as well as activating mutations in the TP53 pathway.[9] Therefore, BIA-ALCL seems multifactorial in its origin, rather than established through a cause-effect linear relationship.

The present study has several limitations that could lead to an underestimation of the exact number of cases in Portugal. First, we only included healthcare institutions with a department of Plastic, Reconstructive and Aesthetic Surgery. This could be a relevant limitation since breast reconstructive surgery is a wide field that can involve various medical specialties such as General Surgery, Gynecology, Hematology or Pathology. Even though most patients that underwent reconstructive techniques involving breast implants are theoretically followed by plastic surgeons, we cannot ignore the potential existence of BIA-ALCL cases outside this department. The inclusion of Pathology departments, which normally have access to their own patient databases or of Hematology departments may be a crucial point in obtaining reliable estimates and should be considered in future studies that attempt to estimate the prevalence of this rare type of lymphoma. On the other hand, it would be interesting to analyze the total number of breast implants placed *per* institution in order to determine the frequency of this rare event. However, obtaining a reliable and accurate numerator (number of cases of BIA-ALCL in Portugal) is a critical step to properly assess the risk of BIA-ALCL in further studies. Furthermore, our sample included 28 private institutions, but the total number of private healthcare centers where breast cosmetic surgery is performed is markedly higher. Nevertheless, BIA-ALCL is a rare and novel entity and, thus, it is expectable that those cases are referred to specialized, large dimension and experienced centers, such as IPO. All specialized centers were included in this study, thus overcoming this limitation. BIA-ALCL usually develops on average nine years after implantation. In contrast with patients who underwent reconstructive surgery after breast cancer, cosmetic surgery patients seldom follow-up with their plastic surgeon for longer than one year post-surgery.[13] Hence, the absence of cases in some healthcare institutions can simply reflect an incomplete follow-up. Moreover, other medical specialties less aware of BIA-ALCL may delay its diagnosis or even misdiagnose it, when confronted with symptomatic patients in the emergency department.[13] The fear of litigation and the absence of a validated national registry are also potential variables that could contribute to underestimating our results.

Even though our study only identified one confirmed case of BIA-ALCL, several hospitals reported having late-onset seromas that did not match the required criteria to be classified as BIA-ALCL after pathological examination. Seromas that arise more than one year after implantation occur in approximately 0.1% - 0.2% of patients and it is estimated that BIA-ALCL occurs in 9% - 13% of such cases.[9] Some authors propose that BIA-ALCL may originate in a pre-existing lymphoproliferative disorder characterized by an indolent localized (*in situ*) disease that resolves in most cases with capsulectomy and implant removal.[24] The National Comprehensive Cancer Network (NCCN) recommends that symptomatic peri-prosthetic effusions that develop more than one year after implantation should be aspirated and screened for CD30 in immunohistochemistry and flow cytometry.[10] Nonetheless, as mentioned previously, BIA-ALCL can also present as a mass without a coexisting seroma, regional lymphadenopathy or skin lesions,[6] demanding a high index of suspicion.

BIA-ALCL is a rare disease which generally follows an indolent course. Most cases resolve with implant removal and complete capsulectomy. However, 15% of cases correspond to more advanced stages of the disease and require treatment with chemotherapy, antibody-drug conjugates (such as brentuximab vedotin) or both.[20] These modalities of treatment carry high long-term morbidity due to their systemic toxicity. For women who undergo breast reconstructive surgery after malignancy or as a prophylactic measure, the risk regarding BIA-ALCL, even though low, might be unacceptable. Currently, the prophylactic removal of textured prosthesis is not recommended as BIA-ALCL remains an extremely rare and mostly curable disease.[1]

After implant removal and complete capsulectomy in the context of BIA-ALCL, the reconstruction of the breast should be executed with autologous tissue or smooth implants.[21] Furthermore, the NCCN claims that surgeons might consider the removal of the contralateral implant since 4.6% of cases were identified as having an incidental lymphoma in the contralateral breast.[10]

Based on the hypotheses that a subclinical infection might be in the origin of BIA-ALCL, the use of techniques that minimize the bacterial load at the time of surgery, specifically the 14-point plan, might reduce the occurrence of this disease. Adams et al designed a prospective study to test this possibility, following eight plastic surgeons that were asked to perform the 14 point-plan.[25] After the implementation of this technique in women with macrotextured breast implants, no cases of BIA-ALCL were reported, although the expected number according to previous Australian studies would be between eight and nine.[25]

The number of cases of BIA-ALCL has increased considerably in the last 10 years.[26] The increasing use of macrotextured implants, improved awareness and the time-lag required for the development of the disease are some of the factors that might contribute to this upward trend.[26] Nevertheless, it is difficult to establish a reliable determination due to the dispersion of data and single reports.

The adherence to registries and mandatory reporting are the most important vehicles towards adequate surveillance, tracking, and detailed epidemiological profiling.[3] Various countries have already created voluntary national registries concerning BIA-ALCL. In 2012, the American Society of Plastic Surgeons, The Plastic Surgery Foundation and FDA created PROFILE. This patient registry represented a systematic tool to collect and unify data concerning patients diagnosed with BIA-ALCL in the United States.[23] Similarly, the Netherlands has a mandatory registry since 2016.[27] Portugal should also create a national registry regarding BIA-ALCL in order to accurately clarify the epidemiology of the disease in our country.

Patients in the cosmetic field rarely follow-up with their plastic surgeon more than one year post-surgery. However, BIA-ALCL develops on average nine years after implantation. Extending the follow- up of these patients would probably carry a heavy burden on public healthcare systems for a largely curable and exceptionally rare disease. Future clinical practice should focus on informing women with breast implants about the alarming signs of the disease that should motivate the return to their plastic surgeon. In our opinion, all women should be fully informed regarding the type of implant they have and should perform regular self-breast exams. Moreover, other specialties should be educated and made aware about BIA-ALCL, allowing for its recognition both in the emergency department and primary care settings.

BIA-ALCL may represent a shift for surgeons regarding selection of implant type. Smooth implants or autologous tissue represent adequate alternatives for women who wish to undergo a cosmetic or reconstructive procedure. Women should be fully informed about the potential risks and advantages of textured implants and consent to the alternative that is more acceptable for them. Furthermore, manufacturers should offer new alternatives to implant design, materials and surface texture.[4]

CONCLUSION

Despite its rarity, BIA-ALCL remains a potentially fatal disease. The current use of textured implants should be reviewed and the potential harms concerning their application must be weighted in medical decisions.

This study was the first to attempt to bring some light to the epidemiological pattern of BIA-ALCL in Portugal. In the future, prospective, large-dimension national studies and the creation of national patient registries could bring additional and relevant information about the impact of this disease in the national playground.

AUTHORS CONTRIBUTION

AC: Data acquisition, draft of the manuscript.
RH: Data acquisition and critical review.
DB: Data acquisition.

DISCLAIMER

This dissertation was presented as a summary in oral communication in “XLIX Reunião Anual da Sociedade Portuguesa de Cirurgia Plástica, Reconstrutiva e Estética” (Nov 7-Nov 9 2019 in Fundação Cupertino de Miranda, Porto, Portugal) and was never published in a scientific journal.

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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 2013 Helsinki Declaration of the World Medical Association.

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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REFERENCES

1. Cardoso MJ, Wyld L, Rubio IT, Leidenius M, Curigliano G, Cutuli B, et al. EUSOMA position regarding breast implant associated anaplastic large cell lymphoma (BIA-ALCL) and the use of textured implants. Breast. 2019;44:90–3.
2. American Society of Plastic Surgeons. Plastic Surgery Statistics Report. 2018 [cited 2019 Sep 15]. Available from: https://www.plasticsurgery.org/documents/News/Statistics/2018/plastic-surgery-statistics-full-report-2018.pdf.
3. Collett DJ, Rakhorst H, Lennox P, Magnusson M, Cooter R, Deva AK. Current risk estimate of breast implant-associated anaplastic large cell lymphoma in textured breast implants. Plast Reconstr Surg. 2019;143:S30-40.
4. Dixon JM, Clemens M. Breast implants and anaplastic large cell lymphoma. BMJ. 2018;363:k5054.
5. Swanson E. Plastic surgeons defend textured breast implants at 2019 U.S. Food and Drug Administration Hearing: why it is time to reconsider. Plast Reconstr Surg Glob Open. 2019;7:e2410.
6. O’Neill AC, Zhong T, Hofer S. Implications of breast implant-associated anaplastic large cell lymphoma (BIA-ALCL) for breast cancer reconstruction: an update for surgical oncologists. Ann Surg Oncol. 2017;24:3174–9.
7. Ebner P, Liu A, Gould D, Patel K. Breast implant–associated anaplastic large cell lymphoma, a systematic review and in-depth evaluation of the current understanding. J Surg Oncol. 2019;120:573-7.
8. Adrada B, Miranda R, Rauch G, Arribas E, Kanagal-Shamanna R, Clemens MW, et al. Breast implant-associated anaplastic large cell lymphoma: sensitivity, specificity, and findings of imaging studies in 44 patients. Breast Cancer Res Treat. 2014;147:1–14.

9. Rastogi P, Deva A. Breast implant-associated anaplastic large cell lymphoma. HM Curr Hematol Malig Rep. 2018;13:516–24.
10. Clemens MW, Horwitz SM. NCCN consensus guidelines for the diagnosis and management of breast implant-associated anaplastic large cell lymphoma. Aesthetic Surg J. 2017;37:285–9.
11. Food and Drug Administration. Medical device reports of breast implant-associated anaplastic large cell lymphoma. 2019. [cited 2020 Jul 14]. Available from: https://www.fda.gov/medical-devices/breast-implants/medical-device-reports-breast-implant-associated-anaplastic-large-cell-lymphoma.
12. Brody GS, Deapen D, Taylor CR, Pinter-Brown L, House-Lightner SR, Andersen JS, et al. Anaplastic large cell lymphoma occurring in women with breast implants: analysis of 173 cases. Plast Reconstr Surg. 2015;135:695–705.
13. Leberfinger AN, Behar BJ, Williams NC, Rakszawski KL, Potochny JD, MacKay DR, et al. Breast implant-associated anaplastic large cell lymphoma: a systematic review. JAMA Surg. 2017;152:1161–8.
14. Doren EL, Miranda RN, Selber JC, Garvey PB, Liu J, Medeiros LJ, et al. U.S. epidemiology of breast implant-associated anaplastic large cell lymphoma. Plast Reconstr Surg. 2017;139:1042–50.
15. Myckatyn TM, Parikh RP. Discussion: breast implant–associated anaplastic large cell lymphoma in Australia and New Zealand: high-surface-area textured implants are associated with increased risk. Plast Reconstr Surg. 2017;140:655–8.
16. Hu H, Johani K, Almatroudi A, Vickery K, Van Natta B, Kadin ME, et al. Bacterial biofilm infection detected in breast implant-associated anaplastic large-cell lymphoma. Plast Reconstr Surg. 2016;137:1659–69.
17. Brinton LA. The relationship of silicone breast implants and cancer at other sites. Plast Reconstr Surg. 2007;120:S94–102.
18. Kellogg BC, Hiro ME, Payne WG. Implant-associated anaplastic large cell lymphoma. Ann Plast Surg. 2014;73:461–4.
19. Agence Nationale de Sécurité du Médicament et des Produits de Santé.. Décision du 02/04/2019 portant interdiction de mise sur le marché, de distribution, de publicité et d'utilisation d'implants mammaires à enveloppe macro-texturée et d'implants mammaires polyuréthane, ainsi que retrait de ces produits.. 2019. [cited 2019 Feb 15]. Available from: https://ansm.sante.fr/Decisions/Injonctions-decisions-de-police-sanitaire-sanctions-financieres-interdictions-de-publicite-Decisions-de-police-sanitaire/Decision-du-02-04-2019-portant-interdiction-de-mise-sur-le-marche-de-distribution-de-publicite-et-d-utilisation-d-implants-mammaires-a-enveloppe-macro-texturee-et-d-implants-mammaires-polyurethane-ainsi-que-retrait-de-ces-produits.
20. Myckatyn TM, Mehta-Shah N, Duncavage E. Breast implant–associated anaplastic large cell lymphoma: real, rare, but avoidable. JAMA Surg. 2019 (in press). doi: 10.1001/jamasurg.2019.3154,
21. Groth AK, Graf R. Breast implant-associated anaplastic large cell lymphoma (BIA-ACLL) and the textured breast implant crisis. Aesth Plast Surg. 2020;44:1–12.
22. Loch-Wilkinson A, Beath K, Knight R, Wessels W, Magnusson M, Papadopoulos T, et al. Breast implant-associated anaplastic large cell lymphoma in Australia and New Zealand: high-surface-area textured implants are associated with increased risk. Plast Reconstr Surg. 2017;140:645–54.
23. McCarthy CM, Loyo-Berríos N, Qureshi AA, Mullen E, Gordillo G, Pusic AL, et al. Patient registry and outcomes for breast implants and anaplastic large cell lymphoma etiology and epidemiology (PROFILE): initial report of findings, 2012-2018. Plast Reconstr Surg. 2019;143:S65-73.
24. Kadin ME, Adams WP, Inghirami G, Di Napoli A. Does breast implant associated ALCL begin as a lmphoproliferative disorder? Plast Reconstr Surg. 2020;145:30e-8.
25. Adams W, Culbertson E, Deva A, Magnusson M, Layt C, Jewell M, et al. Macrotextured breast implants with defined steps to minimize bacterial contamination around the device: experience in 42 000 implants. Plast Reconstr Surg. 2017;140:427–31.
26. Santanelli di Pompeo F, Sorotos M. EURAPS Editorial: BIA-ALCL, a brief overview. J Plast Reconstr Aesthetic Surg. 2018;71:785–7.
27. Adler N. Breast implant-associated anaplastic large cell lymphoma: solid data or fake news? Isr Med Assoc J. 2019;21:516.

Prevalence of Cytogenetic Abnormalities and *FMR1* Gene Premutation in a Portuguese Population with Premature Ovarian Insufficiency

Prevalência de Anomalias Citogenéticas e da Pré-Mutação do Gene *FMR1* numa População Portuguesa com Insuficiência Ovárica Prematura

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ABSTRACT

Introduction: Chromosome abnormalities contribute to about 10% of cases of premature ovarian insufficiency. Most are associated with X chromosome. *Fragile mental retardation 1* (*FMR1*) gene premutation has an estimated prevalence of 1% - 7% in sporadic cases and up to 13% in familial cases. Our aim was to describe the clinical characteristics, cytogenetic and *FMR1* testing of a Portuguese population with premature ovarian insufficiency.

Material and Methods: Women diagnosed with premature ovarian insufficiency in a Portuguese tertiary centre were retrospectively analysed. Data were retrieved from electronic medical records including clinical characteristics, cytogenetic and *FMR1* testing. The main outcome measures were the prevalence of chromosome abnormalities and *FMR1* premutation in a Portuguese population with premature ovarian insufficiency.

Results: Ninety-four patients were included, with a median age at menopause of 36 years. The prevalence of chromosome abnormalities was 16.5% (14/85) and most were X chromosome related (78.6%, n = 11). The prevalence of *FMR1* premutation was 6.7% (6/90). The prevalence of karyotypic abnormalities or *FMR1* premutation did not differ significantly between familial and sporadic cases. Neither chromosome abnormalities nor *FMR1* premutation influenced age at menopause or follicle stimulating hormone levels at diagnosis in premature ovarian insufficiency patients.

Discussion: This is the first study describing the clinical characteristics and both cytogenetic and *FMR1* testing in a Portuguese population with premature ovarian insufficiency. The rate of chromosome abnormalities in our sample was higher than in other populations, while the prevalence of *FMR1* premutation was similar to previous reports.

Conclusion: Our results underline the importance of genetic screening in premature ovarian insufficiency patients in both etiological study and genetic counselling.

Keywords: Chromosome Abnormalities; Cytogenetic Analysis; Fragile X Mental Retardation Protein; Premature Ovarian Insufficiency

RESUMO

Introdução: As anomalias cromossómicas contribuem para 10% dos casos de insuficiência ovárica prematura estando maioritariamente associadas ao cromossoma X. A pré-mutação do gene *fragile mental retardation 1* (*FMR1*) tem uma prevalência estimada de 1% - 7% nos casos esporádicos e até 13% nos casos familiares. O nosso objetivo foi descrever as características clínicas e a análise citogenética e do gene *FMR1* de uma população Portuguesa com insuficiência ovárica prematura.

Material e Métodos: Análise retrospetiva das mulheres com o diagnóstico de insuficiência ovárica prematura vigiadas num hospital terciário Português. Recolha de dados através do processo médico eletrónico incluindo características clínicas, análise citogenética e análise do gene *FMR1*. Os desfechos principais foram a prevalência de anomalias cromossómicas e da pré-mutação *FMR1* numa população Portuguesa com insuficiência ovárica prematura.

Resultados: Foram incluídas 94 doentes, com uma mediana de idade de menopausa de 36 anos. A prevalência de anomalias cromossómicas foi 16,5% (14/85) e a maioria estavam relacionadas com o cromossoma X (78,6%, n = 11). A prevalência da pré-mutação *FMR1* foi de 6,7% (6/90). A prevalência de anomalias cromossómicas ou pré-mutação *FMR1* não diferiu entre casos esporádicos e familiares. Nem as anomalias cromossómicas nem a pré-mutação *FMR1* influenciaram a idade de menopausa ou os níveis da hormona estimulante dos folículos aquando do diagnóstico na população com insuficiência ovárica prematura.

Discussão: Este é o primeiro estudo a descrever as características clínicas e a análise citogenética e do gene *FMR1* numa população Portuguesa com insuficiência ovárica prematura. A prevalência de anomalias cromossómicas na nossa amostra foi superior à descrita para outras populações, enquanto a prevalência da pré-mutação *FMR1* foi semelhante à descrita em estudos anteriores.

Conclusão: Os nossos resultados sublinham a importância do rastreio genético em doentes com insuficiência ovárica prematura, quer no estudo etiológico, quer no aconselhamento genético.

Palavras-chave: Análise Citogenética; Anomalias Cromossómicas; Insuficiência Ovárica Prematura; Proteína do X Frágil de Retardo Mental

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INTRODUCTION

Premature ovarian insufficiency (POI) is defined as the loss of ovarian function before the age of 40 and affects approximately 1% of women.[1] Clinically, patients may present with primary or secondary amenorrhea, or with oligomenorrhea.[1] Several factors have been recognized as causes of POI, such as genetic factors, previous chemo- or radiotherapy, bilateral ovarian surgery, autoimmune or infectious diseases.[1–3] In most cases, however, the underlying cause will remain unknown.

In the last decades, an increasing interest has been drawn to the genetic causes of POI.[4–6] Chromosome abnormalities are known to be present in 10% - 13% of patients with POI and most are associated with the X chromosome.[4,7,8] Numerous karyotypic abnormalities have been reported, ranging from X chromosome deletions, X-autosome translocations or X-isochromosomes to numerical defects.[4,9] X-monosomy, both with and without mosaicism, has been associated with an accelerated follicular atresia.[4] Previous studies have reported that 47,XXX patients are also at risk for POI, with a prevalence varying between 1.5% and 3.8%. The exact mechanism is still unclear but an association with autoimmune diseases or a meiotic disturbance caused by an extra X chromosome have been proposed.[4,7,10] In 1973, Sarto *et al* defined a X chromosome critical region from Xq13-Xq21 to Xq23-q27.[11] The implication of this region in translocations or deletions was associated with POI. Multiple studies have corroborated this finding.[4]

The *fragile mental retardation 1* (*FMR1*) gene is the strongest genetic association with POI.[6] The *FMR1* premutation (*FMR1*-PM) has a prevalence of 1:130 - 1:250 in the female population.[5,12] Carriers of premutated alleles, with 55 - 200 CGG repeats, are known to have a risk of developing POI as high as 34%.[13,14] An association between the number of CGG repeats and the development of POI has been reported, although the number of repeats associated with the highest risk is still a matter of debate.[15–17] Contrary to what has been reported in the past, intermediate alleles (45 - 54 CGG repeats) do not seem to be associated with POI.[18]

Carriers of *FMR1*-PM are not only at risk of developping POI, but also have an increased risk of fragile-X-associated tremor/ataxia syndrome (FXTAS).[1,19] This is a late onset neurodegenerative disorder, characterized by gait ataxia, dementia and intention tremor, which occurs in male carriers of *FMR1*-PM. The penetrance of symptoms increases with age, affecting more than one third of patients over 50 years of age and exceeding 50% for men aged 70 - 90 years. Females are also affected although to a lesser extent.[19]

Another reason to test for *FMR1*-PM is the increased risk of expanding to the full length mutation (over 200 CGG repeats) in the offspring, leading to the Fragile X Syndrome (FXS). This risk is directly associated with the number of the premutation carrier CGG repeats, increasing significantly with more than 65 - 70 repeats.[20]

These figures highlight the importance of the genetic characterization of these patients, both at the chromosomal and molecular level. This will contribute to a better understanding of the biological mechanisms associated with POI. Moreover, this knowledge will allow for an evaluation of their family risk of developing POI or having a fragile X or FXTAS descendent, identifying family members candidates for genetic evaluation, genetic counseling or prenatal diagnosis. In this regard, a multidisciplinary approach envolving gynecologists, obstetricians, geneticists and neurologists is of paramount importance in the correct counselling of these patients.

It is known that population characteristics, such as ethnicity, may affect POI prevalence and its genetic contribution.[1] Therefore, our aim was to describe both cytogenetic abnormalities and *FMR1* tests in a Portuguese population with POI.

MATERIAL AND METHODS

Study design

Our group carried out a retrospective study regarding patients with the diagnosis of POI who attended their first visit in a tertiary university-affiliated hospital between January 2010 and December 2018. The study was performed in accordance with the 2013 Helsinki Declaration and with approval of the Institutional Ethics Committee (reg. 010-2020). Since the study involved completely anonymous data extraction from electronic medical records, patient consent was not required. The inclusion criteria were: primary or secondary idiopathic amenorrhea for at least four months in women under 40 years old and two serum follicle stimulating hormone (FSH) measurements over 25 mUI/mL obtained at least one month apart. Patients with conditions known to induce POI (previous chemo- or radiotherapy, ovarian surgery and autoimmune diseases) were excluded. Patients with typical Turner syndrome stigmata were also ruled out. Family history of POI was considered when a history of first or second-degree relatives with POI was present. Family history of Fragile X syndrome was validated when a medical report confirming the diagnosis was available.

Electronic medical records were reviewed for gynecological and obstetric history (age at menarche and menopause, gravidity and parity, previous miscarriages and menstrual pattern), family history of POI and fragile X syndrome and laboratory results (plasma serum FSH and estradiol levels at diagnosis, cytogenetic analysis and *FMR1* test).

FSH and estradiol measurements

Plasma serum levels of estradiol and FSH were measured using a commercial chemiluminescence array (CMIA) using the Architect analyser (Abbot Diagnostics, Spain).

Cytogenetic analysis

Chromosomal analysis was performed on metaphases obtained from 72 h phytohemagglutinin (PHA) stimulated peripheral blood lymphocyte cultures according to standard procedures. Analysis of GTG-banded chromosomes was

done at a resolution of 700 bands per haploid genome, according to the International System for Human Cytogenetic Nomenclature (ISCN) 2016.[21] A minimum of 30 cells were counted to rule out mosaicism, the common occurrence of age related sex chromosome losses and/or gains was considered before reporting sex chromosome mosaicism.[22,23]

FMR1 testing

Genomic DNA was extracted from peripheral blood lymphocytes using Jetquick blood and cell culture DNA Midi Spin kit (Genomed, Löhne, Germany) and DNA concentration and purity were evaluated using a NanoDrop1000 Spectrophotometer (Thermo Scientific, Waltham, USA). *FMR1* gene CGG repeat number was determined by conventional PCR using primers C and F described by Fu *et al* and by Triplet Repeat Primed PCR (TP PCR) using Asuragen AmplideX® *FMR1* PCR Kit (Asuragen, Austin, USA), as previously described by Ferreira *et al*.[24,25]

Statistical analysis

Statistical analysis was performed using SPSS Statistics, Version 23.0 (IBM Corp., Armonk, NY, USA). Categorical variables were compared using the Fisher's exact test according to the Cochrane rules. Quantitative non-normal variables were expressed as median (interquartile range) and the non-parametric Mann-Whitney U test was used for distribution comparisons. All tests were 2 tailed, and $p < 0.05$ was considered statistically significant.

RESULTS

Clinical characteristics

A total of 94 patients enrolled the study. Patients' gynecological and family history is shown in Table 1. Median age at menopause was 36.0 (6.0) years. The majority of patients reported secondary amenorrhea (95.7%, n = 90).

Obstetric history was unavailable in four patients. Overall, the nulliparity rate was 40.0% (36/90) and 18.9% (17/90) of the patients had a history of previous spontaneous miscarriage.

Twenty-three patients presented a family history of POI.

Table 1 – Patients' baseline characteristics

Obstetric and gynecological history	
Age at menarche (years)	12.0 (3.0)
Primary amenorrhea	4/94 (4.3%)
Nulligravida	31/90 (34.4%)
Nullipara	36/90 (40.0%)
Previous miscarriage	17/90 (18.9%)
Age at menopause (years)	36.0 (6.0)
FSH at diagnosis (IU/L)	79.0 (43.9)
Estradiol at diagnosis (pg/mL)	20.0 (10.0)
Family history	
POI	23/94 (24.4%)
Fragile X syndrome	2/94 (2.1%)

Values are median (interquartile range) deviation or n (%)

The prevalence of primary amenorrhea was 4.3% (1/23) in familial cases and 4.2% (3/71) in sporadic POI patients. A family history of fragile X syndrome was present in 2 patients. None of the cases with family history of fragile X syndrome presented with primary amenorrhea.

No statistically significant difference was found between the median FSH at diagnosis in patients with primary *versus* secondary amenorrhea [64.9 (56.0) *vs* 80.0 (39.0) IU/L, $p = 0.392$, Mann Whitney test].

Chromosomal abnormalities

Due to missing data, the karyotype was analysed in 85 patients (Table 2).

An abnormal karyotype was observed in 16.5% (n = 14), of which 78.6% (n = 11) involved the X chromosome. The most common abnormality was X chromosome mosaicism, which was found in 50.0% of our cohort (7/14). The four patients with primary amenorrhea presented a normal karyotype.

No statistically significant differences were found regarding age at menopause [35.5 (7.8) *vs* 36.0 (6.0) years, $p = 0.691$, Mann Whitney test] or FSH at diagnosis [83.0 (62.0) *vs* 78.1 (32.0) IU/L, $p = 0.415$, Mann Whitney test] between patients with (n = 14) or without (n = 71) an abnormal karyotype.

Also, no statistically significant difference was found regarding the prevalence of karyotypic abnormalities between the 23 patients with a family history of POI (8.7%, n = 2) and those without (19.4%, n = 12) ($p = 0.333$, Fisher's exact test).

FMR1 testing

Due to missing data, *FMR1* analysis was performed in 90 patients (Table 3). *FMR1*-PM was present in 6.7%

Table 2 – Karyotyping

Normal (46, XX)	**71/85** (83.5%)
Abnormal	**14/85** (16.5%)
X chromosome related	
- 46,X,del(X)(q25~q26).ish del(X)(DXYS61-)	
- 46,X,del(X).ish del(X)(pter-q22.2)(DXS28-)	
- 46,X,t(X;8)(q24;q24.22)	
- 47,XXX	
- mos 45,X[2]/46,XX[28].nuc ish(DXZ1x1)[4/110]	
- mos 45,X[1]/47,XXX[1]/48,XXXX[1]/46,XX[47]	
- mos 45,X[3]/47,XXX[1]/46,XX[26]	
- mos 45,X[3]/47,XXX[1]/46,XX[26]	
- mos 45,X[3]/47,XXX[1]/46,XX[16]	
- mos 47,XXX[3]/45,X[1]/46,XX[26]	
- mos 47,XXX[2]/45,X[1]/46,XX[32]	
Non-X chromosome related	
- mos 47,XX,+21[2]/46,XX[38]	
- 45,XXder(13;14)(q10;q10)	
- 47,XX,+mar.ishder(14/22)(D14Z1/D22Z1+,D22S75-)	

Table 3 – Number of CGG repeats in *FMR1* testing

Normal alleles	**82 patients/90** (91.1%)
< 29	62
29	18
30	53
31	19
32 - 44	19
Intermediate zone alleles	**2 patients/90** (2.2%)
53	1
54	1
Premutation alleles	**6 patients/90** (6.7%)
58	1
60	2
69	1
80	1
82	1

(n = 6). The most frequent CGG number of repeats was 30 (n = 53), followed by 31 (n = 19) and 29 (n = 18).

None of the four patients with primary amenorrhea presented the *FMR1*-PM.

No significant difference was found between patients with and without *FMR1*-PM concerning age at menopause [38.0 (1.8) *vs* 36.0 (6.0) years, p = 0.092, Mann Whitney test] or FSH levels at diagnosis [84.7 (63.0.) *vs* 77.7 (40.0) IU/L, p = 0.340, Mann Whitney test].

There was a higher prevalence of *FMR1*-PM in patients with a family history of POI, but this difference was not statistically significant [13.0% (3/23), *vs* 4.5% (3/67), p = 0.176, Fisher's exact test].

Both patients with a family history of X fragile syndrome carried premutated alleles [(30,60) and (35,58)].

DISCUSSION

This is the first study describing the clinical characteristics and both cytogenetic and *FMR1* testing in a Portuguese population with POI.

The median age at menopause in our population was 36 years, similar to the results published by Murray *et al* in a UK population.[26] In an Italian study, Baronchelli *et al* also reported a mean age at menopause of 34 years.[27] However, in this study the authors considered patients with menopause before the age of 45 years.[27] Janse *et al* described a median age at menopause of 32 years in a POI Dutch population.[28] Lower ages at menopause have been reported in POI non-European populations, varying between 24 and 30 years.[7,8,29–31] Although more studies are needed to consolidate this data, the available evidence seems to point towards a higher age at menopause in European populations with POI. This is in line with previous reports which suggest differences regarding age of natural menopause in different ethnic groups.[32] Despite the controversy regarding race/ethnicity *per se* as a factor that influences age at menopause, a higher educational level, the prolonged use of oral contraceptives and a higher baseline weight seem to be associated with a higher age at natural menopause.[32,33] The exact mechanism behind these associations is not completely understood. Although no epidemiological studies have been performed in POI populations, we hypothesize that these factors may also contribute to our results.

The prevalence of primary amenorrhea in our population was 4.3% (95% CI 1.6% - 11.0%), which is lower than in other populations (13.2% - 51.0%).[7,8,28,29,31,34] We hypothesized that the fact that our department attends to predominantly adult patients might have contributed to this bias.

The rate of previous spontaneous miscarriage was 18.9% (95% CI 12.0% - 28.5%), which is higher than the findings reported by Allen *et al* and Jansel *et al* in a POI population (5.0% - 13.9%), but similar to the expected rate in the general population.[15,28,35]

The prevalence of chromosomal abnormalities in our population was 16.5% (95% CI 9.9% - 26.1%). Most studies report a prevalence of karyotypic abnormalities varying between 9% and 14%.[7,8,27,28,31,34] However, a higher prevalence, between 21% and 32%, has also been reported in Tunisian, American, Chilean and Turkish populations.[29,36–38] Similarly to what has been previously published, most karyotypic abnormalities were X chromosome related.[7,8,28,29] In our population, in accordance with the results of Lakahl *et al* and Janse *et al*, the most frequent were mosaic numerical X chromosome abnormalities.[28,31] Other authors reported X chromosome structural abnormalities as being the most frequent.[7,8,27,29] Regarding X chromosome structural abnormalities, in our sample, all cases involved the Xq, which is in agreement with previous studies and with the critical regions previously defined for the development of POI (Xq13-Xq21 and Xq23-Xq27).[4,7,11] Two patients presented Robertsonian translocations, which have also been previously reported in POI patients, although the autosomal role in POI remains unexplained.[4,7] Finally, one patient, who was referred to our department due to secondary amenorrhea, presented one autosomal mosaic involving trisomy 21 in two different cell lines [47,XX,+21(2)/46,XX(38)]. Being a mosaic, we cannot predict the presence of the trisomy in other tissues and a causal effect with POI cannot be excluded, since women with Down syndrome have a higher chance of suffering from POI.[39,40]

Despite the fact that previous studies found a higher prevalence of chromosome abnormalities in patients with primary amenorrhea than in patients with secondary amenorrhea,[8,29,31] none of the cases with primary amenorrhea presented karyotypic abnormalities in our study. Most certainly, the small size of the primary amenorrhea subgroup (n = 4) was underpowered to detect these differences. Similarly to what has been previously described, no difference was found regarding the prevalence of karyotypic abnormalities in familial and sporadic cases.[8,31]

The prevalence of *FMR1*-PM in our sample was 6.7% (96% CI 3.0% - 14.2%), similar to what has been previously described in non-Asian populations.[1,29] In Asian populations, the prevalence seems to be lower (0.5% - 1.5%).[30]

This is in line with a previous study involving almost 135 000 women from an unselected pan-ethnic cohort, which also reported a lower incidence of *FMR1*-PM in Asian patients.[41] In accordance with other studies, the prevalence was higher in familial cases of POI (13.6% *vs* 4.5%).[5,14,26] The fact that this difference was not statistically significant in our sample may also be attributed to the small sample size. The number of CGG repeats has emerged as a possible predictor of risk and severity of *FMR1*-related POI. Despite still being a matter of debate, 80 - 100 repeat alleles seem to confer the highest risk.[15,16] In our sample, among the six patients with *FMR1*-PM, only two presented alleles in the high-risk zone (respectively, 80 and 82).

The most frequent number of CGG repeats has been reported as 32,[4] while in our sample the most frequent allele was 30. This probably reflects population related variations, which account for the importance of the genetic characterization of these patients on a population level. Considering that fragile X syndrome is the result of the expansion of the number of CGG repeats when transmitted from mother to offspring, the fact that both patients with family history of fragile X syndrome presented *FMR1*-PM [(30,60) and (35,58)] was an expected finding. In accordance with the findings by Bouali *et al*, all patients with *FMR1*-PM presented with secondary amenorrhea.[29]

CONCLUSION

Taking into account the prevalence of chromosomal abnormalities and *FMR1*-PM in our cohort, these results demonstrate the importance of genetic screening for patients with POI and add new data on the different phenotypic and genotypic patterns of this disorder in different populations. We highlight the higher prevalence of chromosome abnormalities in our Portuguese cohort. Chromosomal studies and *FMR1* testing not only provide an etiological explanation for the POI patient, but they also bear important information for both reproductive and genetic counselling, both for the couple and other relatives. Taking into account the extra-reproductive risks conferred by *FMR1*-PM, namely FXS and FXTAS, the importance of a multidisciplinary approach for these patients, involving gynaecologists, obstetricians, neurologists and medical geneticists should not be disregarded.

AUTHORS CONTRIBUTION

ARN: Concept and design of the work; literature review; data acquisition, analysis and interpretation; draft of the paper.

ASP, SIF, AE, EM: Data acquisition and interpretation; critical review of the manuscript; approval of the final version.

VR, FA: Data interpretation; critical review of the manuscript; approval of the final version.

MJC: Concept and design of the work; data acquisition and interpretation; critical review of the manuscript; approval of the final version.

FG, IMC: Concept and design of the work; data interpretation; critical review of the manuscript; 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 2013 Helsinki Declaration of the World Medical Association.

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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REFERENCES

1. European Society for Human Reproduction and Embryology (ESHRE) Guideline Group on POI, Webber L, Davies M, Anderson R, Bartlett J, Braat D, Cartwright B, et al. ESHRE Guideline: management of women with premature ovarian insufficiency. Hum Reprod. 2016;31:926-37.
2. Coccia ME, Rizzello F, Mariani G, Bulletti C, Palagiano A, Scarselli G. Ovarian surgery for bilateral endometriomas influences age at menopause. Hum Reprod. 2011;26:3000-7.
3. Silva C, Yamakami L, Aikawa N, Araujo D, Carvalho J, Bonfá E. Autoimmune primary ovarian insufficiency. Autoimmun Rev. 2014;13:427–30.
4. Qin Y, Jiao X, Simpson JL, Chen ZJ. Genetics of primary ovarian insufficiency: New developments and opportunities. Hum Reprod Update. 2015;21:787-808.
5. Rossetti R, Ferrari I, Bonomi M, Persani L. Genetics of primary ovarian insufficiency. Clin Genet. 2017;91:183-98.
6. Greene AD, Patounakis G, Segars JH. Genetic associations with diminished ovarian reserve: A systematic review of the literature. J Assist Reprod Genet. 2014;31:935-46.
7. Jiao X, Qin C, Li J, Qin Y, Gao X, Zhang B, et al. Cytogenetic analysis of 531 Chinese women with premature ovarian failure. Hum Reprod. 2012;27:2201-7.
8. Kalantari H, Madani T, Zari Moradi S, Mansouri Z, Almadani N, Gourabi H, et al. Cytogenetic analysis of 179 Iranian women with premature ovarian failure. Gynecol Endocrinol. 2013;29:588-91.
9. Liu J, Cox L. Primary ovarian insufficiency: an update. Int J Womens Health. 2014;6:235.
10. Goswami R, Goswami D, Kabra M, Gupta N, Dubey S, Dadhwal V. Prevalence of the triple X syndrome in phenotypically normal women with premature ovarian failure and its association with autoimmune thyroid disorders. Fertil Steril. 2003;4:1052–4.
11. Sarto G, Therman E, Patau K. X inactivation in man: a woman with t(Xq--;12q+). Am J Hum Genet. 1973;3:262–70.
12. Hagerman P. The fragile X prevalence paradox. J Med Genet. 2008;45:498-9.
13. Utine GE, Şimşek-Kiper PÖ, Akgün-Doğan Ö, Ürel-Demir G, Alanay Y, Aktaş D, et al. Fragile x-associated premature ovarian failure in a large

Turkish cohort: Findings of Hacettepe Fragile X Registry. Eur J Obstet Gynecol Reprod Biol. 2018;221:76-80.
14. Wittenberger MD, Hagerman RJ, Sherman SL, McConckie-Rosell A, Welt C, Rebar RW, et al. The FMR1 premutation and reproduction. Fertil Steril. 2007;87:456-65.
15. Allen E, Sullivan A, Marcus M, Small C, Dominguez C, Epstein MP, et al. Examination of reproductive aging milestones among women who carry the FMR1 premutation. Hum Reprod. 2007;22:2142-52.
16. Sullivan AK, Marcus M, Epstein MP, Allen EG, Anido AE, Paquin JJ, et al. Association of FMR1 repeat size with ovarian dysfunction. Hum Reprod. 2005;20:402-12.
17. Spath M, Feuth T, Smits A, Yntema HG, Braat DD, Thomas CM, et al. Predictors and risk model development for menopausal age in fragile x premutation carriers. Genet Med. 2011;13:643-50.
18. Schufreider A, McQueen DB, Lee SM, Allen R, Uhler M, Davie J, et al. Diminished ovarian reserve is not observed in infertility patients with high normal CGG repeats on the fragile X mental retardation 1 (FMR1) gene. Hum Reprod. 2015;30:2686-92.
19. Jacquemont S, Hagerman RJ, Hagerman PJ, Leehey MA. Fragile-X syndrome and fragile X-associated tremor / ataxia syndrome : two faces of FMR1. Lancet Neurol. 2007;6:45-55.
20. Yrigollen C, Durbin-Johnson B, Gane L, Nelson DL, Hagerman R, Hagerman PJ, et al. AGG interruptions within the maternal FMR1 gene reduce the risk of offspring with fragile X syndrome. Genet Med. 2012;14:729-36.
21. McGowan-Jordan J, Simons A, Schmid M, editors. An international system for human cytogenomic nomenclature. Basel: S. Karger; 2016.
22. Gardner R, Sutherland G, Shaffer L. Chromosome abnormalities and genetic counselling. 4th ed. Oxford: Oxford University Press; 2012.
23. Guttenbach M, Koschorz B, Bernthaler U, Grimm T, Schmid M. Sex chromosome loss and aging: in situ hybridisation studies on human interphase nuclei. Am J Hum Genet. 1995;57:1143-50.
24. Fu Y, Kuhl D, Pizzuti A, Pieretti M, Sutcliffe JS, Richards S, et al. Variation of the CGG repeat at the fragile X site results in genetic instability: resolution of the Sherman paradox. Cell. 1991;67:1047-58.
25. Ferreira SI, Pires LM, Ferrão J, Sá J, Serra A, Carreira IM. Mosaicism for FMR1 gene full mutation and intermediate allele in a female foetus: a postzygotic retraction event. Gene. 2013;527:421-5.
26. Murray A, Schoemaker MJ, Bennett CE, Ennis S, MacPherson JN, Jones M, et al. Population-based estimates of the prevalence of FMR1 expansion mutations in women with early menopause and primary ovarian insufficiency. Genet Med. 2014;16:19-24.
27. Baronchelli S, Conconi D, Panzeri E, Bentivegna A, Redaelli S, Lissoni S, et al. Cytogenetics of premature ovarian failure: an investigation on 269 affected women. J Biomed Biotechnol. 2011;2011:20-2.
28. Janse F, Knauff E, Niermeijer M, Eijkemans MJ, Laven JS, Lambalk CB, et al. Similar phenotype characteristics comparing familial and sporadic premature ovarian failure. Menopause. 2010;17:758-65.
29. Bouali N, Hmida D, Mougou S, Bouligand J, Lakhal B, Dimessi S, et al. Analysis of FMR1 gene premutation and X chromosome cytogenetic abnormalities in 100 Tunisian patients presenting premature ovarian failure. Ann Endocrinol. 2015;76:671-8.
30. Guo T, Qin Y, Jiao X, Li G, Simpson JL, Chen ZJ. FMR1 premutation is an uncommon explanation for premature ovarian failure in Han Chinese. PLoS One. 2014;9:6-10.
31. Lakhal B, Braham R, Berguigua R, Bouali N, Zaouali M, Chaieb M, et al. Cytogenetic analyses of premature ovarian failure using karyotyping and interphase fluorescence in situ hybridization (FISH) in a group of 1000 patients. Clin Genet. 2010;78:181-5.
32. Gold EB. The timing of the age at which natural menopause occurs. Obs Gynecol Clin N Am. 2011;38:425-40.
33. Gold EB, Crawford SL, Avis NE, Crandall CJ, Matthews KA, Waetjen LE, et al. Original contribution factors related to age at natural menopause : longitudinal analyses from SWAN. Am J Epidemiol. 2013;178:70-83.
34. Portnoï MF, Aboura A, Tachdjian G, Bouchard P, Dewailly D, Bourcigaux N, et al. Molecular cytogenetic studies of Xq critical regions in premature ovarian failure patients. Hum Reprod. 2006;21:2329-34.
35. National Collaborating Centre for Women's and Children's Health (UK). Ectopic pregnancy and miscarriage: ectopic pregnancy and miscarriage: diagnosis and initial management in early pregnancy of ectopic pregnancy and miscarriage. 2012. [accessed 2019 Jan 15]. Available rom: https://www.nice.org.uk/guidance/cg154/.
36. Ceylaner G, Altinkaya S, Mollamahmutoglu L, Ceylaner S. Genetic abnormalities in Turkish women with premature ovarian failure. Int J Gynaecol Obstet. 2010;110:122-4.
37. Castillo S, López F, Tobella L, Salazar S, Daher V. The cytogenetics of premature ovarian failure. Rev Chil Obs Ginecol. 1992;57:341-5.
38. Rebar R, Connolly H. Clinical features of young women with hypergonadotropic amenorrhea. Fertil Steril. 1990;53:804-10.
39. Romualdi D, Proto C, De Cicco S, Immediata V, Barone C, Romano C, et al. Low AMH levels as a marker of reduced ovarian reserve in young women affected by Down's syndrome. Menopause. 2016;23:1247-51.
40. Parizot E, Dard R, Janel N, Vialard F. Down syndrome and infertility: what support should we provide? J Assist Reprod Genet. 2019;36:1063-1067.
41. Owens KM, Dohany L, Holland C, DaRe J, Mann T, Settler C, et al. FMR1 premutation frequency in a large, ethnically diverse population referred for carrier testing. Am J Med Genet Part A. 2018;176:1304-8.

MYOC Gene Sequencing Analysis in Primary Open-Angle Glaucoma Patients from the Centre Region of Portugal

Análise por Sequenciação do Gene *MYOC* em Doentes com Glaucoma Primário de Ângulo Aberto da Região Centro de Portugal

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ABSTRACT

Introduction: Primary open-angle glaucoma is the most frequent subtype of glaucoma. Relatives of primary open-angle glaucoma patients have an increased risk of developing the disease, suggesting a genetic predisposition to the disease. *MYOC* was the first primary open-angle glaucoma-causing gene identified. This study aimed to identify sequence variations in the *MYOC* gene that may be responsible for the phenotype in a group of primary open-angle glaucoma patients from the Centre Region of Portugal.

Material and Methods: The three coding exons and the proximal splicing junctions of the *MYOC* gene were studied using a PCR-sequencing approach in a group of 99 primary open-angle glaucoma patients.

Results: The sequencing analysis enabled the identification of 20 variants, including four in the promoter region, seven in the introns and nine in exons one and three, of which four were missense variants.

Discussion: Initially, all four missense sequence variations identified were considered candidates to glaucoma causing disease mutations. However, after literature review, only variant c.1334C>T (Ala445Val) remained as likely responsible for mild late-onset normal tension glaucoma.

Conclusion: This is the first study performed in a group of primary open-angle glaucoma patients from the Centre Region of Portugal, contributing to the identification of one genetic variant in the *MYOC* gene and reinforcing the hypothesis that normal tension glaucoma could be also due to *MYOC* gene mutations.

Keywords: Genetics; Glaucoma/diagnosis; Low Tension Glaucoma; Mutation, Missense

RESUMO

Introdução: O glaucoma primário de ângulo-aberto é o subtipo mais frequente de glaucoma. Os familiares de doentes com glaucoma primário de ângulo-aberto têm um risco maior de desenvolverem a doença, o que sugere uma predisposição genética para a doença. *MYOC* foi o primeiro gene causador de glaucoma primário de ângulo-aberto a ser identificado. Este estudo pretendeu identificar variações de sequência no gene *MYOC* que possam ser responsáveis pelo fenótipo num grupo de doentes com glaucoma primário de ângulo-aberto da Região Centro de Portugal.

Material e Métodos: Os três exões codificantes e as regiões adjacentes do gene *MYOC* foram estudados utilizando o método de PCR-sequenciação num grupo de 99 doentes com glaucoma primário de ângulo aberto.

Resultados: A análise de sequenciação permitiu identificar 20 variantes, incluindo quatro na região promotora, sete nos intrões e nove nos exões um e três, das quais quatro eram variantes missense.

Discussão: Inicialmente, todas as quatro variações de sequência missense identificadas foram consideradas candidatas a mutações causadoras de glaucoma. No entanto, após análise da literatura, somente a variante c.1334C>T (Ala445Val) permaneceu como provável responsável pelo glaucoma de pressão normal de início tardio.

Conclusão: Este é o primeiro estudo realizado num grupo de doentes com glaucoma primário de ângulo aberto da Região Centro de Portugal, contribuindo para a identificação de uma variante genética no gene *MYOC* e reforçando a hipótese de que o glaucoma de pressão normal também poderá ser causado por mutações no gene *MYOC*.

Palavras-chave: Genética; Glaucoma/diagnóstico; Glaucoma de Baixa Pressão; Mutação Missense

INTRODUCTION

Glaucoma is a group of optic neuropathies essentially characterized by a progressive degeneration of retinal ganglion cells (RGCs) and their axons, leading to excavation in the optic nerve head and, consequently, characteristic, progressive and irreversible visual field defects.[1] At the beginning, the peripheral vision loss may not interfere with the daily routine and remains undetected.[2] Therefore, until an advanced stage of the disease is reached, which usually consists on central vision loss, most patients are unaware that they have the disease and, consequently, remain undiagnosed and untreated.[3]

This ocular disease is the second leading cause of blindness[4] and the leading cause of irreversible blindness in the world, affecting 67 million people, of which 85% - 90%

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have primary open-angle glaucoma (POAG) in developed countries.[5-6] It is estimated that the number of people with glaucoma in the world will increase to 76 million in 2020 and to 111.8 million in 2040, from which 52.7 million and 79.8 million, respectively, will be POAG patients.[7]

Although the pathogenesis of POAG has not been fully elucidated, there are known risk factors for the disease including elevated intraocular pressure (IOP), age, ethnicity, a positive family history, pseudoexfoliation, central corneal thickness, myopia and ocular perfusion pressure.[4,6] And even though elevated IOP is a risk factor for POAG, it is not a necessary feature for diagnosis since an important subtype of POAG, known as normal tension glaucoma (NTG), occurs at low to normal IOP levels.[6] NTG accounts for approximately 20% - 50% of all POAG cases.[8]

There is little doubt that a positive family history increases the risk of developing glaucoma. Relatives of POAG patients have been shown to have an eight-fold increase in the disease risk,[4,6] exhibiting an autosomal dominant heredity.[9] *MYOC*, a gene composed by three exons and encoding a protein with 504 amino acids,[10] was the first POAG-causing gene identified[11] and mutations in this gene are responsible for approximately 2% to 4% of the cases.[12] Prior to the identification of this gene, Sunden *et al* mapped the *GLC1A locus* comprising an interval in the long arm of chromosome 1 (1q21-q31), that was associated with juvenile open-angle glaucoma (JOAG), a subtype of POAG with onset earlier than 35 years old (yo) and very high IOP.[13] Afterwards, this region was limited to chromosome 1q23 - q25. The defective gene in this locus was finally identified as *TIGR* (*Trabecular meshwork-Inducible Glucocorticoid Response*).[11] In the meantime, Japanese researchers mapped this gene by FISH within the chromosome 1q23 - 1q24 region, and due to its homologous regions with myosin, *TIGR* was named *MYOC.*[14]

The myocilin protein is ubiquitously expressed in normal tissues and organs,[15] widely expressed in ocular tissues and highly expressed in the trabecular meshwork (TM), where it plays an important role in the regulation of IOP.[16-18] Despite a number of studies over a 20 year period since its discovery in 1997, the physiological functions and biological activities of myocilin in the TM remain poorly understood. Aggregation of aberrant mutant myocilins is closely associated with glaucoma pathogenesis. The aggregation of misfolded/wild-type myocilins in the endoplasmic reticulum (ER) may be harmful for TM cells and lead to apoptosis.[18] Previous results have suggested that the TM is several times thicker in patients with glaucoma harboring mutations compared with that in patients without myocilin mutations. Therefore, myocilin mutations appear to be involved in the morphological changes in the TM, which lead to cell apoptosis.[19]

The present study aimed to identify sequence variations in the *MYOC* gene that may be responsible for the phenotype in a group of POAG patients from the Centre Region of Portugal.

MATERIAL AND METHODS

Human subjects

A group of 99 Portuguese Caucasian POAG patients from the Centre Region of Portugal, consisting of 52 males and 47 females with an average age of 71.2 yo and ranging from 42 to 88 yo, and an average age at diagnosis of 61.7 yo and ranging from 23 to 82 yo, was recruited to this study and a clinical characterization was performed at the Ophthalmology Department of the Centro Hospitalar e Universitário de Coimbra. All patients underwent a detailed ophthalmologic examination to ensure the diagnosis of POAG including: 1) exclusion of secondary causes, 2) open drainage angles on gonioscopy (Shaffer's grading III-IV), 3) presence of typical glaucomatous optic disc damage (excavation) and 4) visual field defects detected by automated perimetry (with Humphrey's perimeter). The IOP was also evaluated since ocular tension enables the distinction of POAG subtypes. Accordingly, glaucoma patients with IOP equal or below 21 mmHg are diagnosed as NTG.[4] For the present study, 26 patients with high IOP and 73 with IOP equal or below 21 mmHg (NTG) were randomly recruited.

This study was approved by the Ethics Committee of the Faculty of Medicine, University of Coimbra, following the tenets of the Declaration of Helsinki 2013 and a written consent for genetic testing was obtained from adult probands.

Sequence variations identification

The DNA was extracted from the peripheral blood of POAG patients using a standard phenol–chloroform method followed by ethanol precipitation.[20]

Individual exons and adjacent regions of the *MYOC* gene were amplified by polymerase chain reaction (PCR) using primers designed with Primer3 software (http://bioinfo.ut.ee/primer3-0.4.0/primer3/). The primers nucleotide sequence will be made available upon request to the corresponding author. The PCR reactions were performed using 50 ng of genomic DNA mixed with the following reagents: 1X Taq Buffer 10X [with $(NH_4)_2SO_4$] (Fermentas), 0.2 µM of forward and reverse primers (Sigma-Aldrich), 1.5 mM $MgCl_2$ (Fermentas), 0.2 mM dNTPs (5PRIME), 1U Taq Polymerase (Fermentas) and RNAse/DNase free Water (AccuGENE) to a final volume of 10 µl. The reaction mixtures were subjected to a specific PCR program with an initial denaturation step of five minutes (min) at 95ºC followed by 35 cycles, each with denaturation at 95°C for 30 seconds (sec), annealing at 59ºC - 63°C for 30 sec, and extension for one min at 72°C, with a final elongation step of 10 min at 72°C.

PCR products underwent an electrophoresis on an agarose gel containing 1% agarose SeaKem LE (Lonza) and 1% ethidium bromide (Acros/Fisher bioreagents) in 1X Tris Borate EDTA (TBE) solution (National diagnostics).

The amplified PCR products were purified using 1 µl of ExoSAP-IT® and sequencing reactions were performed using BigDye® Terminator v3.1 according to manufacturer recommendations (Applied Biosystems) and the primers

previously used for target DNA amplification. The sequencing reaction products were separated on a Genetic Analyzer 3130 (Applied Biosystems) equipment. DNA sequencing data obtained was analysed using Sequencing Analysis Software v.5.4® (Applied Biosystems) and SeqScape v2.5® (Applied Biosystems).

RESULTS

After PCR-sequencing analysis of the 99 POAG Portuguese patients it was possible to identify at least one genetic variant in *MYOC* gene exons or in the adjacent regions in all the patients but two. Overall, 20 different sequence variations were found, including nine coding and 11 non-coding variants (Table 1). From the coding variants four were missense alterations [c.227G>A p.(Arg76Lys), c.878C>A p.(Thr293Lys), c.1193A>G p.(Lys398Arg) and c.1334C>T p.(Ala445Val) (Fig. 1)] and five were synonymous variants [c.39T>G p.(Pro13Pro), c.141C>T p.(Cys47Cys), c.477A<G p.(Leu159Leu), c.975G>A p.(Thr325Thr) and c.1041T>C p.(Tyr347Tyr)]. Four non-coding sequence variations were found in the promoter region (c.-224T>C, c.-190G>T, c.-126T>C and c.-83G>A),

Table 1 – Sequence variations identified in *MYOC* gene of POAG patients

Sequence variation	Amino acid change	Genotype (n = 99)		
		Wild type homozygous	Heterozygous	Mutant homozygous
c.-224T>C	-	54 TT	41 TC	4 CC
c.-190G>T	-	98 GG	1 GT	-
c.-126T>C	-	94 TT	5 TC	-
c.-83G>A	-	83 GG	16 GA	-
c.39T>G	p.(Pro13Pro)	96 TT	3 TG	-
c.141C>T	p.(Cys47Cys)	97 CC	2 CT	-
c.227G>A	p.(Arg76Lys)	83 GG	16 GA	-
c.477A>G	p.(Leu159Leu)	96 AA	3 AG	-
c.604+50G>A	-	97 GG	2 GA	-
c.605-332G>A	-	71 GG	26 GA	2 AA
c.605-280G>T	-	16 GG	57 GT	26 TT
c.605-210delT	-	97 TT	2 Tdel	-
c.730+35A>G	-	45 AA	46 AG	8 GG
c.731-205A>C	-	66 AA	32 AC	1 CC
c.731-73C>T	-	95 CC	4 CT	-
c.878C>A	p.(Thr293Lys)	98 CC	1 CA	-
c.975G>A	p.(Thr325Thr)	96 GG	3 GA	-
c.1041T>C	p.(Tyr347Tyr)	94 TT	5 TC	-
c.1193A>G	p.(Lys398Arg)	97 AA	2 AG	-
c.1334C>T	**p.(Ala445Val)**	**98 CC**	**1 CT** *	**-**

* Marks the individual with the variant responsible for late-onset NTG.
The nomenclature used in Table 1 for sequence variations is according to reference.[45]

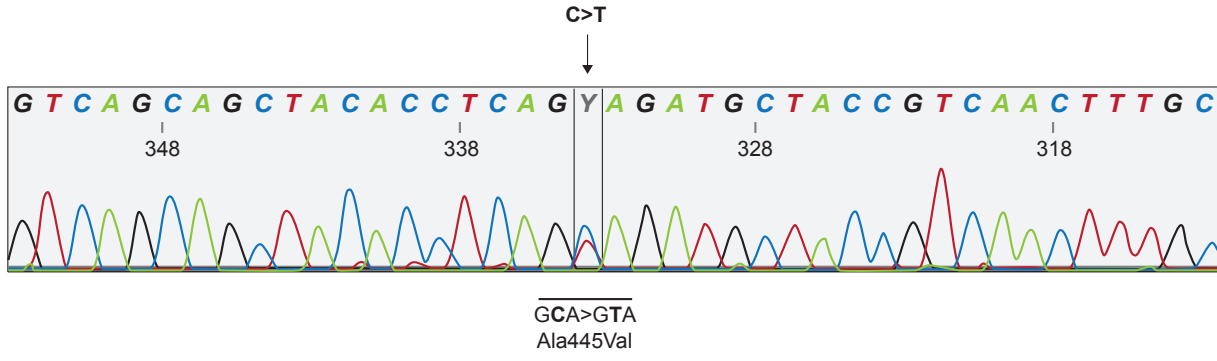

Figure 1 – Electropherogram of the genetic region surrounding variant c.1334C>T p.(Ala445Val) identified in a normal tension glaucoma patient. Heterozygous transition of a C to a T at nucleotide 1334, changing the codon GCA to GTA and causing an Alanine to Valine amino acid substitution at position 445. The nomenclature used in Fig. 1 for sequence variation is according to reference.[45]

four were found in intron one (c.604+50G>A, c.605-332G>A, c.605-280G>T and c.605-210delT) and three were found in intron two (c.730+35A>G, c.731-205A>C and c.731-73C>T) (Table 1). The missense variants c.878C>A p.(Thr293Lys) and c.1334C>T p.(Ala445Val) (Fig. 1) were identified in heterozygosity in 1 patient each, the c.1193A>G p.(Lys398Arg) in two patients and variant c.227G>A p.(Arg76Lys) was identified in 16 patients (Table 1). It is noticeable that the promoter variant c.-83G>A and the exon one missense alteration c.227G>A p.(Arg76Lys) were always found simultaneously in the same patients, even sharing the same genotype.

DISCUSSION

The sequencing analysis of the *MYOC* gene in 99 POAG patients allowed the identification of 20 variants including four missense alterations [c.227G>A p.(Arg76Lys), c.878C>A p.(Thr293Lys), c.1193A>G p.(Lys398Arg) and c.1334C>T p.(Ala445Val) (Fig. 1)] (Table 1). Initially, all four missense sequence variations were considered candidates to glaucoma causing disease mutations. However, after literature review, it was possible to determine that c.227G>A p.(Arg76Lys), c.878C>A p.(Thr293Lys) and c.1193A>G p.(Lys398Arg) variants were previously described in individuals without the glaucoma phenotype,[21-28] thus likely neutral polymorphisms. Nevertheless, the variant c.1334C>T p.(Ala445Val) (Fig. 1) was previously identified only in glaucoma patients and consequently reported as a glaucoma causing mutation.[8,22,29-33]

With the aim of developing a biochemical assay to distinguish different forms of myocilin protein, a cellular assay with Triton X-100 detergent was applied to determine protein solubility of mutant and normal forms of the protein, taking into consideration that misfolded myocilin mutants aggregate in the ER and are insoluble. Variant c.1193A>G p. (Lys398Arg) was one of the studied variants and the assay established its solubility,[34] and consequently non-pathogenicity.

Upon crystal structure-based prediction, variant p.(Thr293Lys) is a remote surface exposed residue having wild-type-like stability, which makes it unlikely to promote misfolding of myocilin protein.[35] Considering this data and the identification of c.878C>A p. (Thr293Lys) in individuals without the glaucoma phenotype, this variant is most likely a neutral polymorphism.

Variant c.1334C>T p. (Ala445Val) (Fig. 1) is located in *MYOC* gene exon 3 and results from an alteration at the second nucleotide of codon 445, changing an amino acid alanine to a valine. Even if it is unlikely that this amino acid change causes alterations in the protein properties since both alanine and valine are non-polar and hydrophobic amino acids,[29] the strong former α alanine changes to a strong former β valine may possibly cause an increased preference for a β-sheet conformation[36] and a significant modification in the secondary structure of the myocilin protein. However, based on crystal structure-based prediction, p. (Ala445Val) is located on a remote surface of olfactomedin (OLF) domain[35] and exhibits wild-type-like stability for OLF melting temperature,[37] suggesting that it is not prone to misfolding.[35] Nevertheless, using size exclusion chromatography, it was possible to determine that the p. (Ala445Val) OLF domain has a higher yield of aggregated species, allowing the identification of significant differences in the ratio of aggregate to monomer species when compared with wild type OLF domain, suggesting that p.(Ala445Val) is more similar to disease causing variants than to the wild-type.[37] Additionally, in the present study, the c.1334C>T p.(Ala445Val) (Fig. 1) variant was identified in a male NTG patient with 77 yo at diagnosis and an IOP of 17mm/Hg in both eyes. Taking into consideration that *MYOC* gene mutations are mainly associated with JOAG patients with an early onset before 35 yo and very high IOP, most likely caused by severe morphological changes in TM, our study suggests that the c.1334C>T p.(Ala445Val) variant may be responsible for a mild late onset form of glaucoma regardless of TM dysfunction and likely caused by a neurodegenerative mechanism affecting RGC, as previously proposed.[38] Finally, the present study supports the glaucoma causing mutation classification for the *MYOC* variant c.1334C>T p.(Ala445Val) based on its association with late onset NTG and high yield of aggregated species.

Additional results obtained in the present study include four non-coding sequence variations found in the *MYOC* gene promoter region (c.-224T>C, c.-190G>T, c.-126T>C and c.-83G>A) (Table 1). As much as it was possible to determine from the literature review, there is no association between the *MYOC* gene promoter region variants and any type of glaucoma, and so it is still questionable if these variants may influence *MYOC* gene expression and lead to glaucoma. Also interesting for future line of research is the simultaneous identification of the promoter variant c.-83G>A and exon 1 missense alteration c.227G>A p.(Arg76Lys), even sharing the same genotype in every patient and suggesting a segregation in linkage disequilibrium as previously reported.[32,39-42] If separately both are unanimously considered neutral polymorphisms for glaucoma, their impact in linkage remains elusive.

Personalized medicine using genetic information to anticipate disease onset and progression, and to implement preventive interventions for each patient is an evolving field.[43] This is directly associated with the exponential drop in cost of high-throughput genome-wide genotyping platforms.[44] Next generation, high-throughput DNA sequencing technology offers a powerful approach to identify causal genetic variants for many rare and common genetic disorders, including POAG.[10] Genetic testing for POAG is clearly helpful in some specific situations, such as screening of family members in autosomal dominant POAG of early onset.[44] Early identification of mutation carrying individuals creates the opportunity for early implementation of medical and surgical treatment alternatives for slowing down the progression or even preventing glaucoma from developing.[43] But this is only possible if genetic testing is included in the diagnostic criteria for glaucoma. Recently,

it was demonstrated that *MYOC* cascade genetic testing for POAG allows identification of at-risk individuals at an early stage or even before signs of glaucoma are present.[43] This was only possible due to *MYOC* gene screening and identification of disease-causing mutations in POAG patients and further mutation screening in patients' relatives. Without genetic testing as a diagnostic criterion there will be no mutation identification, no relatives tested, no early diagnosis achieved and no preventive therapies applied. Taking into consideration the present study, gathering of DNA samples for genetic testing from relatives of the patient with variant c.1334C>T p.(Ala445Val) is ongoing.

CONCLUSION

This is the first study performed in a group of POAG patients from the Centre Region of Portugal contributing to the identification of one genetic variant in the *MYOC* gene [c.1334C>T p.(Ala445Val)], probably responsible for a mild late onset glaucoma through a neurodegenerative mechanism that is independent of TM dysfunction. These findings will enable cascade genetic testing of patient's relatives with the aim of identifying at-risk individuals and implementing therapeutic procedures to prevent the development of glaucoma. Accordingly, genetic testing should be included in the diagnostic approach for glaucoma.

AUTHORS CONTRIBUTION

FS: Substantial contribution to the conception, design of the work, acquisition analysis and interpretation of data for the work. Drafting and final approval of the version to be published.

FF: Substantial contribution to the acquisition, analysis and interpretation of data for the work. Drafting the work and revising it critically for important intellectual content. Final approval of the version to be published.

PF: Substantial contribution to the conception, acquisition and interpretation of data for the work. Revising it critically for important intellectual content. Final approval of the version to be published.

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JP, JFS: Substantial contribution to the acquisition of data for the work. Revising it critically for important intellectual content. Final approval of the version to be published.

MG, HG. PP: Substantial contribution to the conception of the work. Revising it critically for important intellectual content. Final approval of the version to be published.
JMP: Substantial contribution to the conception and acquisition of data for the work. Revising it critically for important intellectual content. Final approval of the version to be published.

All authors agreed 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 2013 Helsinki Declaration of the World Medical Association.

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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REFERENCES

1. Fuse N. Genetic bases for glaucoma. Tohoku J Exp Med. 2010;22:1-10.
2. Janssen SF, Gorgels TG, Ramdas WD, Klaver CC, van Duijn CM, Jansonius NM, et al. The vast complexity of primary open angle glaucoma: disease genes, risks, molecular mechanisms and pathobiology. Prog Retin Eye Res. 2013;37:31-67.
3. Fan BJ, Wiggs JL. Glaucoma: genes, phenotypes, and new directions for therapy. J Clin Invest. 2010;120:3064-72.
4. European Glaucoma Society. Chapter 2 – Classification and Terminology. In: European Glaucoma Society, editors. Terminology and Guidelines for Glaucoma. 4th ed. Savona: PubliComm; 2014. p.79-87.
5. Hong CJ, Trope GE. Glaucoma. CMAJ. 2015;187:E398-9.
6. Youngblood H, Hauser MA, Liu Y. Update on the genetics of primary open-angle glaucoma. Exp Eye Res. 2019;188:107795.
7. Tham YC, Li X, Wong TY, Quigley HA, Aung T, Cheng CY. Global prevalence of glaucoma and projections of glaucoma burden through 2040: a systematic review and meta-analysis. Ophthalmology. 2014;121:2081-90.
8. Weisschuh N, Neumann D, Wolf C, Wissinger B, Gramer E. Prevalence of myocilin and optineurin sequence variants in German normal tension glaucoma patients. Mol Vis. 2005;11:284-7.
9. Fingert JH. Primary open-angle glaucoma genes. Eye. 2011;25:587-95.
10. Liu Y, Allingham RR. Major review: molecular genetics of primary open-angle glaucoma. Exp Eye Res. 2017;160:62-84.
11. Stone EM, Fingert JH, Alward WL, Nguyen TD, Polansky JR, Sunden SL, et al. Identification of a gene that causes primary open angle glaucoma. Science. 1997;275:668-70.
12. Hulsman CA, De Jong PT, Lettink M, van Duijn C, Hofman A, Bergen AA. Myocilin mutations in a population-based sample of cases with

open angle glaucoma: the Rotterdam Study. Graefes Arch Clin Exp Ophthalmol. 2002;240:468-74.
13. Sunden SL, Alward L, Nichols BE, Rokhlina TR, Nystuen A, Stone EM, et al. Fine mapping of the autosomal dominant juvenile open angle glaucoma (GLC1A) region and evaluation of candidate genes. Genome Res. 1996;6:862-9.
14. Kubota R, Noda S, Wang Y, Minoshima S, Asakawa S, Kudoh J, et al. A novel myosin-like protein (myocilin) expressed in the connecting cilium of the photoreceptor: molecular cloning, tissue expression, and chromosomal mapping. Genomics. 1997;41:360-9.
15. Borrás T. The effects of myocilin expression on functionally relevant trabecular meshwork genes: a mini-review. J Ocul Pharmacol Ther. 2014;30:202-12.
16. Jain A, Zode G, Kasetti RB, Ran FA, Yan W, Sharma TP, et al. CRISPR-Cas9-based treatment of myocilin-associated glaucoma. Proc Natl Acad Sci U S A. 2017;114:11199-204.
17. Hernandez H, Millar JC, Curry SM, Clark AF, McDowell CM. BMP and activin membrane bound inhibitor regulates the extracellular matrix in the trabecular meshwork. Invest Ophthalmol Vis Sci. 2018;59:2154-66.
18. Wang H, Li M, Zhang Z, Xue H, Chen X, Ji Y. Physiological function of myocilin and its role in the pathogenesis of glaucoma in the trabecular meshwork (Review). Int J Mol Med. 2019;43:671-81.
19. Hamanaka T, Kimura M, Sakurai T, Ishida N, Yasuda J, Nagasaki M, et al. A histologic categorization of aqueous outflow routes in familial open-angle glaucoma and associations with mutations in the MYOC gene in Japanese patients. Invest Ophthalmol Vis Sci. 2017;58:2818-31.
20. Preparation and analysis of DNA. In: Ausubel FM, Brent R, Kingston RE, Moore DD, Seidman JG, Smith JA, et al, editors. In: Current protocols in molecular biology. New York: John Wiley & Sons, Inc; 2003. p. 1–12.
21. Izumi K, Mashima Y, Obazawa M, Ohtake Y, Tanino T, Miyata H, et al. Variants of the myocilin gene in Japanese patients with normal-tension glaucoma. Ophthalmic Res. 2003;35:345-50.
22. Faucher M, Anctil JL, Rodrigue MA, Duchesne A, Bergeron D, Blondeau P, et al. Founder TIGR/myocilin mutations for glaucoma in the Québec population. Hum Mol Genet. 2002;11:2077-90.
23. Jia LY, Tam PO, Chiang SW, Ding N, Chen LJ, Yam GH, et al. Multiple gene polymorphisms analysis revealed a different profile of genetic polymorphisms of primary open-angle glaucoma in northern Chinese. Mol Vis. 2009;15:89-98.
24. Tanwar M, Kumar M, Dada T, Sihota R, Dada R. MYOC and FOXC1 gene analysis in primary congenital glaucoma. Mol Vis. 2010;16:1996-2006.
25. Whigham BT, Williams SE, Liu Y, Rautenbach RM, Carmichael TR, Wheeler J, et al. Myocilin mutations in black South Africans with POAG. Mol Vis. 2011;17:1064-9.
26. Banerjee D, Bhattacharjee A, Ponda A, Sen A, Ray K. Comprehensive analysis of myocilin variants in east Indian POAG patients. Mol Vis. 2012;18:1548-57.
27. Liu W, Liu Y, Challa P, Herndon LW, Wiggs JL, Girkin CA, et al. Low prevalence of myocilin mutations in an African American population with primary open-angle glaucoma. Mol Vis. 2012;18:2241-6.
28. Park J, Kim M, Park CK, Chae H, Lee S, Kim Y, et al. Molecular analysis of myocilin and optineurin genes in Korean primary glaucoma patients. Mol Med Rep. 2016;14:2439-48.
29. Alward WL, Fingert JH, Coote MA, Johnson AT, Lerner SF, Junqua D, et al. Clinical features associated with mutations in the chromosome 1 open-angle glaucoma gene (GLC1A). N Engl J Med 1998;338:1022-7.
30. Fingert JH, Héon E, Liebmann JM, Yamamoto T, Craig JE, Rait J, et al. Analysis of myocilin mutations in 1703 glaucoma patients from five different populations. Hum Mol Genet. 1999;8:899-905.
31. Vincent AL, Billingsley G, Buys Y, Levin AV, Priston M, Trope G, et al. Digenic inheritance of early-onset glaucoma: CYP1B1, a potential modifier gene. Am J Hum Genet. 2002;70:448-60.
32. López-Martinez F, López-Garrido MP, Sánchez-Sánchez F, Campos-Mollo E, Coca-Prados M, Escribano J. Role of MYOC and OPTN sequence variations in Spanish patients with primary open-angle glaucoma. Mol Vis. 2007;13:862-72.
33. Souzeau E, Burdon KP, Dubowsky A, Grist S, Usher B, Fitzgerald JT, et al. Higher prevalence of myocilin mutations in advanced glaucoma in comparison with less advanced disease in an Australasian disease registry. Ophthalmology. 2013;120:1135-43.
34. Zhou Z, Vollrath D. A cellular assay distinguishes normal and mutant TIGR/myocilin protein. Hum Mol Genet. 1999;8:2221-8.
35. Donegan RK, Hill SE, Freeman DM, Nguyen E, Orwig SD, Turnage KC, et al. Structural basis for misfolding in myocilin-associated glaucoma. Hum Mol Genet. 2015;24:2111-24.
36. Chou PY, Fasman GD. Empirical predictions of protein conformation. Ann Rev Biochem. 1978;47:251-76.
37. Burns JN, Turnage KC, Walker CA, Lieberman RL. The stability of myocilin olfactomedin domain variants provides new insight into glaucoma as a protein misfolding disorder. Biochemistry. 2011;50:5824-33.
38. Gupta N, Yücel Y. Glaucoma as a neurodegenerative disease. Curr Opin Ophthalmol. 2007;18:110-4.
39. Mabuchi F, Yamagata Z, Kashiwagi K, Tang S, Iijima H, Tsukahara S. Analysis of myocilin gene mutations in Japanese patients with normal tension glaucoma and primary open-angle glaucoma. Clin Genet. 2001;59:263-8.
40. Lam DS, Leung YF, Chua JK, Baum L, Fan DS, Choy KW, et al. Truncations in the TIGR gene in individuals with and without primary open-angle glaucoma. Invest Ophthalmol Vis Sci. 2000;41:1386-91.
41. Pang CP, Leung YF, Fan B, Baum L, Tong WC, Lee WS, et al. TIGR/MYOC gene sequence alterations in individuals with and without primary open-angle glaucoma. Invest Ophthalmol Vis Sci. 2002;43:3231-5.
42. Melki R, Belmouden A, Akhayat O, Brézin A, Garchon HJ. The M98K variant of the OPTINEURIN (OPTN) gene modifies initial intraocular pressure in patients with primary open-angle glaucoma. J Med Genet. 2003;40:842-4.
43. Souzeau E, Tram KH, Witney M, Ruddle JB, Graham SL, Healey PR, et al. Myocilin predictive genetic testing for primary open-angle glaucoma leads to early identification of at-risk individuals. Ophthalmology. 2017;124:303-9.
44. Khawaja AP, Viswanathan AC. Are we ready for genetic testing for primary open-angle glaucoma? Eye. 2018;32:877-83.
45. den Dunen JT, Antonarakis SE. Mutation nomenclature extensions and suggestions to describe complex mutations: a discussion. Hum Mutat. 2000;15:7-12.

GJB2: Frequency of the Less Common Variants in a Sample of the Portuguese Population

GJB2: Frequência das Suas Variantes Menos Comuns numa Amostra da População Portuguesa

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ABSTRACT

Introduction: Sequence variants in the *GJB2* gene account for up to 50% of cases of non-syndromic sensorineural hearing loss in the Caucasian population. In this study, we report the frequency of the less common variants of the *GJB2* gene in a Portuguese sample and compare these frequencies with those of a group of hearing-impaired patients.

Material and Methods: In order to select the less common *GJB2* variants, 147 hearing-impaired patients followed in Centro Hospitalar Universitário de São João were evaluated. Afterwards, the presence of those variants was tested in 360 individuals from Generation 21.

Results: The patient assessment enabled the selection of 11 *GJB2* variants. Of those, 10 were investigated in Generation 21 participants, with only four being detected, in heterozygosity: *p.*Phe83Leu, *p.*Arg127His, *p.*Val153Ile and *p.*Asn206Ser, with the allelic frequencies (95% confidence interval) of 0.14% (0.01% - 0.87%), 0.28% (0.01% - 1.08%), 0.97% (0.43% - 2.04%) and 0.14% (0.01% - 0.88%), respectively. Two variants, *p.*Val37Ile and *p.*Val95Met, were more frequent in the patients' group with statistical significance.

Discussion: Our results allow for the *p.*Arg127His and *p.*Val153Ile variants to comply with polymorphism criteria and support the pathogenicity of *p.*Val37Ile and *p.*Val95Met variants. Moreover, two cases of moderate hearing loss were explained by the *p.*Val37Ile/*p.*Asn206Ser genotype, substantiating both the pathogenicity of such variants and the hypothesis that compound heterozygosity with *p.*Ans206Ser is associated with mild-moderate genotypes.

Conclusion: Understanding the role of the variants is essential in order to provide genetic counselling to patients and their families. We explored a set of uncommon *GJB2* variants that comprised 12% of the hearing-impaired patients in this study, supporting the relevance of their description.

Keywords: Connexin 26; Gene Frequency; Genetic Counselling; GJB2 protein, human; Hearing Loss, Sensorineural

RESUMO

Introdução: As mutações no gene *GJB2* são responsáveis por mais de 50% dos casos de hipoacusia neurossensorial não sindrómica na população caucasiana. Neste estudo, reporta-se a frequência das variantes menos comuns do gene *GJB2* numa amostra da população portuguesa, comparando-se com a dos doentes com hipoacusia seguidos na consulta de Genética.

Material e Métodos: Para seleção das variantes menos frequentes do gene *GJB2*, avaliaram-se 147 doentes com hipoacusia seguidos na consulta de Genética – Doenças Hereditárias do Ouvido do Centro Hospitalar Universitário de São João. A presença dessas variantes foi depois testada em 360 indivíduos da Geração 21.

Resultados: A avaliação dos doentes com hipoacusia permitiu selecionar 11 variantes. Dessas, 10 foram pesquisadas nos indivíduos da Geração 21, identificando-se apenas quatro, em heterozigotia: *p.*Phe83Leu, *p.*Arg127His, *p.*Val153Ile e *p.*Asn206Ser, com frequências alélicas (intervalo de confiança 95%) de 0,14% (0,01% - 0,87%), 0,28% (0,01% - 1,08%), 0,97% (0,43% - 2,04%) e 0,14% (0,01% - 0,88%), respetivamente. Duas variantes, *p.*Val37Ile e *p.*Val95Met, mostraram-se mais frequentes nos doentes com hipoacusia de forma estatisticamente significativa.

Discussão: Estes resultados permitem considerar as variantes *p.*Arg127His e *p.*Val153Ile como polimorfismos e apoiam a patogenicidade das variantes *p.*Val37Ile e *p.*Val95Met. Note-se ainda que dois casos de hipoacusia moderada foram justificados pelo genótipo *p.*Val37Ile/*p.*Asn206Ser, apoiando a patogenicidade de tais variantes e corroborando a hipótese de que heterozigotias compostas com a *p.*Asn206Ser cursam com fenótipo ligeiro-moderado.

Conclusão: O conhecimento da patogenicidade das variantes é fundamental para o aconselhamento genético dos doentes e respetivas famílias. No seu conjunto, as variantes do gene *GJB2* analisadas estavam presentes em 12% dos doentes, reiterando a relevância do seu estudo.

Palavras-chave: Aconselhamento Genético; Conexina 26; Frequência do Gene; Gene GJB2 humano; Perda Auditiva Neurossensorial

INTRODUCTION

Sensorineural hearing loss (SNHL) is one of the most common congenital sensory impairments, affecting approximately one in 500 - 1000 newborns.[1] About 60% of cases of early-onset hearing loss are due to genetic causes, of which 70% are non-syndromic.[2] Non-syndromic sensorineural hearing loss (NS-SNHL) is inherited in an autosomal recessive trait in 80%, but it can also be transmitted in autosomal dominant (15% - 20%), X-linked (2% - 3%), or mitochondrial

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(1%) patterns.[3]

More than one hundred genes are known to be involved in NS-SNHL. Despite the genetic heterogeneity, sequence variants in the *GJB2* gene account for up to 50% of cases of NS-SNHL in several populations.[4] This gene encodes connexin 26, which is the major component of gap junctions in the cochlea and has been implicated in the maintenance of K^+ homeostasis in the inner ear.[1] More than two hundred *GJB2* variants have been reported, most of them considered pathogenic.[5] The *p*.Gly12Valfs*2 (also known as c.35delG) variant is the most common *GJB2* mutation in several SNHL populations, including Portugal.[3,6–9] The *p*.Met34Thr variant, recently classified as pathogenic, has also been found with a high frequency among SNHL patients.[3,9,10] The frequency of the *p*.Gly12Valfs*2 and *p*.Met34Thr mutations in the general Portuguese population has already been estimated.[11] As for the remaining *GJB2* variants, no published studies in the Portuguese population have estimated their carrier rates.

The main purpose of this study was to estimate the frequency of the less common *GJB2* variants in a Portuguese community sample – Generation 21 (G21) – and to compare these frequencies with those of SNHL patients. To select the less common *GJB2* variants, a cohort of SNHL patients was evaluated.

We also compare the allelic frequencies of the G21 sample with those of the European sample of the GnomAD Exome population database.[12]

MATERIAL AND METHODS

SNHL patients

A total of 147 consecutive patients followed in the Hereditary Hearing Loss Genetics Clinic from June 2011 until February 2019, presenting with mild to profound SNHL, were studied for *GJB2* variants. For that purpose, peripheral blood samples were collected. Additionally, the audiological evaluation was performed using auditory brainstem response tests or pure tone audiometry. For each patient, the medical history has been collected to determine the age of onset and hearing loss evolution, family and patient history. Moreover, causes of acquired hearing loss were excluded. The severity of SNHL was classified by the pure tone average (PTA) of 0.5, 1, 2 and 4 kHz thresholds: PTA < 20 dB was defined as normal hearing, 21 - 40 dB as mild SNHL, 41 - 70 dB as moderate SNHL, 71 - 95 dB as severe SNHL and PTA > 95 dB as profound SNHL.

Selection of the less common variants

The evaluation of the SNHL patients' cohort provided the spectrum and frequency of *GJB2* variants in this group. Taking this spectrum as reference, every variant other than *p*.Gly12Valfs*2 (c.35delG) and *p*.Met34Thr was included in our set of less common variants - the target variants of this study. Then, the G21 cohort participants were screened for the selected *GJB2* variants, in order to assess their frequency in this Portuguese community group.

G21 cohort participants

Generation 21 (G21), is a population-based birth cohort of 8647 newborns recruited in the metropolitan area of Porto, Portugal, in 2005 - 2006. The recruitment occurred at all level III public units providing obstetrical and neonatal care. At four years of age, the total cohort was invited to a re-evaluation that occurred between April 2009 and July 2011. For this specific study, 480 of the participants attending the follow-up evaluation were randomly selected to study the carrier rate of the most common variants of the *GJB2* gene. To fit into the general study aim, only children with parents of Portuguese nationality were included. For each child, a sample of buccal mucosa cells was collected with a swab. Of the 480 selected participants, only 360 were included in the study, due to sample quality constraints.

Genetic analysis

In total, 360 DNA samples were completely sequenced and analyzed. Firstly, genomic DNA was extracted from buccal mucosa cells, using a commercial kit (JETQUICK, Genomed). Then *GJB2* gene Sanger sequencing was performed. For that purpose, polymerase chain reaction (PCR) of the *GJB2* exon 2 was performed, using the following specific primer pair - Cx26F: 5´-TCTTTTCCAGAGCAAACCGCC-3´ and Cx26R: 5´-TGAGCACGGGTTGCCTCATC-3´. PCR products purification using AmpureXP® was performed to remove the contaminants. The sequencing analysis of amplified fragments was performed on an automated sequencer (Applied Biosystems® 3730xl DNA Analyzer) and the Applied Biosystems® Sequencing Analysis v.5.4 software was used. The annotation of *GJB2* sequence variants was based on the GenBank cDNA reference sequence NM_004004 and following the standard nomenclature recommended by the Human Genome Variation Society.

All phases of the study complied with the Ethical Principles for Medical Research Involving Human Subjects expressed in the Declaration of Helsinki (World Medical Association, 2013). The study was approved by the Centro Hospitalar Universitário de São João Ethics Committee and a signed informed consent according to the Declaration of Helsinki was obtained from all participants.

Statistical analysis

The statistical analysis was performed using GraphPad® and OpenEpi® software. Confidence intervals (CI) were calculated using the modified Wald method.[13] Differences in the allelic frequencies between the G21 and the SNHL groups were tested by Fisher's exact test, as expected values in some cells of the contingency table were below five and the sample size was small. The chi-square test was used to assess differences between the allelic frequencies of the G21 group and the data from the European sample of the GnomAD Exome population database.[12] A significance level of 0.05 was considered.

RESULTS

GJB2 variants in patients with SNHL

A total of 147 index cases with mild to profound SNHL were studied for the presence of variants in the *GJB2* gene. *p*.Gly12Valfs*2 (also known as c.35delG) and *p*.Met34Thr were the most frequently identified variants and were previously studied by Dória M *et al.*[11] Besides those, other 11 less common variants were identified: *p*.Ile20Met, *p*.Trp24X, *p*.Ile30Val, *p*.Val37Ile, *p*.Phe83Leu, *p*.Val95Met, *p*.Arg127His, *p*.Arg143Gln, *p*.Val153Ile, *p*.Arg184Trp and *p*.Asn206Ser. Together, these 11 less common variants were found in 18 of the 147 families included. The allelic frequencies and genotypes are described in Table 1.

Frequency of the less common variants in the G21 group

A total of 360 individuals from the G21 cohort were studied for the presence of the less common variants identified in patients with SNHL. Due to poor DNA amplification by PCR at the extremities of exon 2 of the *GJB2* gene, the *p*.Ile20Met variant was not possible to study, reducing the total number of variants assessed to ten. For the same reason, some of the G21 samples could not be screened for the presence of *p*.Trp24Ter, *p*.Arg184Trp and *p*.Asn206Ser variants, and hence the total number of subjects screened for these variants was 328, 359 and 352, respectively.

Four out of the ten variants assessed have been identified, in heterozygosity, in individuals from the G21. The allelic frequencies are summarized in Table 1. The *p*.Val153Ile variant was found in seven individuals, indicating a carrier rate of approximately 1 in 51 (1.94%, 95% CI 0.86% - 4.04%). The *p*.Arg127His variant was detected in two more participants, indicating a carrier rate of 1 in 180 (0.56%, 95% CI 0.02% - 2.14%). One individual presented the *p*.Phe83Leu variant, indicating a carrier rate of 1 in 360 (0.28%, 95% CI 0.01% - 1.72%). Likewise, the *p*.Asn206Ser variant was found in one participant, suggesting a carrier rate of 1 in 352 (0.28%, 95% CI 0.01% - 1.76%).

No homozygotes or compound

Table 1 – Results of *GJB2* variants in G21 sample, SNHL patients and European Sample of the GnomAD Exome database and statistical comparison between the groups

GJB2 variant	G21 group	SNHL patients	European sample (GnomAD Exome database)	Statistical comparison	
	Alleles no. / n (Frequency, %) (95% CI)	Alleles no. (Frequency, %) (95% CI) n = 294 chromosomes	Alleles no. / n (Frequency, %) (95% CI)	G21-SNHL Fisher's exact test *p*-value*	G21-European sample Chi-square test *p*-value*
p.Ile20Met	–	1 (0.34) (0.01% - 2.10%)	1 / 112153 (0.0009) (0.0000% - 0.0056%)	–	–
p.Trp24Ter	0 / 656 (0.00) (0.00% - 0.70%)	1 (0.34) (0.01% - 2.10%)	7 / 112334 (0.0062) (0.0027% - 0.0132%)	0.3095	0.8398
p.Ile30Val	0 / 720 (0.00) (0.00% - 0.64%)	1 (0.34) (0.01% - 2.10%)	5 / 112798 (0.0044) (0.0016% - 0.0107%)	0.2899	0.8582
p.Val37Ile	0 / 720 (0.00) (0.00% - 0.64%)	5 (1.70) (0.61% - 4.03%)	150 / 113146 (0.1326) (0.1129% - 0.1556%)	0.0020	0.3267
***p*.Phe83Leu**	**1 / 720 (0.14) (0.01% - 0.87%)**	**2 (0.68) (0.02% - 2.61%)**	**377 / 113688 (0.3316) (0.2998% - 0.3668%)**	**0.2032**	**0.3690**
p.Val95Met	0 / 720 (0.00) (0.00% - 0.64%)	5 (1.70) (0.61% - 4.03%)	4 / 113566 (0.0035) (0.0010% - 0.0094%)	0.0020	0.8735
***p*.Arg127His**	**2 / 720 (0.28) (0.01% - 1.08%)**	**4 (1.36) (0.40% - 3.57%)**	**350 / 113072 (0.3095) (0.2788% - 0.3437%)**	**0.0622**	**0.8784**
p.Arg143Gln	0 / 720 (0.00) (0.00% - 0.64%)	1 (0.34) (0.01% - 2.10%)	NA	0.2899	–
***p*.Val153Ile**	**7 / 720 (0.97) (0.43% - 2.04%)**	**1 (0.34) (0.01% -2 .10%)**	**413 / 113494 (0.3639) (0.3305% - 0.4007%)**	**0.4501**	**0.0072**
p.Arg184Trp	0 / 718 (0.00) (0.00% - 0.64%)	1 (0.34) (0.01% - 2.10%)	0 / 113592 (0.0000) (0.0000% - 0.0041%)	0.2905	–
***p*.Asn206Ser**	**1 / 704 (0.14) (0.01% - 0.88%)**	**2 (0.68) (0.02% - 2.61%)**	**8 / 113466 (0.0071) (0.0033% - 0.0142%)**	**0.2090**	**0.0001**

CI: confidence interval; n: total number of chromosomes assessed; NA: not available.
* A significance level of 0.05 was considered.

heterozygotes were found for any of the variants. The *p*.Trp24Ter, *p*.Ile30Val, *p*.Val37Ile, *p*.Val95Met, *p*.Arg143Gln and *p*.Arg184Trp variants were not identified in the cohort.

Comparison between the G21 group and SNHL patients

We compared the allelic frequencies in the G21 group with those of SNHL patients. The differences in the allelic frequencies between the two groups were statistically significant for the *p*.Val37Ile and the *p*.Val95Met variants ($p = 0.0020$), these variants being present only in the SNHL group. Regarding the other variants, no statistically significant differences were found ($p > 0.05$). The statistical results are presented in Table 1.

Comparison between the G21 group and the European sample of the GnomAD Exome

We compared the G21 group allelic frequencies with those of the GnomAD Exome for a (non-Finnish) European sample.[12] The differences were statistically significant for *p*.Val153Ile ($p = 0.0072$) and *p*.Asn206Ser ($p < 0.0001$) variants, with these variants being more frequent in the G21 sample. No statistically significant differences were found for any of the other variants ($p > 0.05$). The allelic frequencies of the *GJB2* variants in the GnomAD European sample, as well as the statistical results, are presented in Table 1.

Characterization of *p*.Phe83Leu, *p*.Arg127His, *p*.Val153Ile and *p*.Asn206Ser variants in families with NS-SNHL

The *p*.Phe83Leu, *p*.Arg127His, *p*.Val153Ile and *p*.Asn206Ser variants correspond the ones that were identified in the G21 sample. In order to further characterize them, we report on the NSHL patients and respective families in which they were present, corresponding to a total of seven unrelated families. The families' genograms with the individuals' genotypes and phenotypes are presented in Fig. 1.

Syndromic hearing loss was evaluated for all hearing-impaired members. Subjects presenting other clinical findings beyond hearing loss were further studied using complementary diagnostics tests and were observed by other medical specialists when required. No individual fulfilled the criteria for syndromic hearing loss. Also, none of them com-

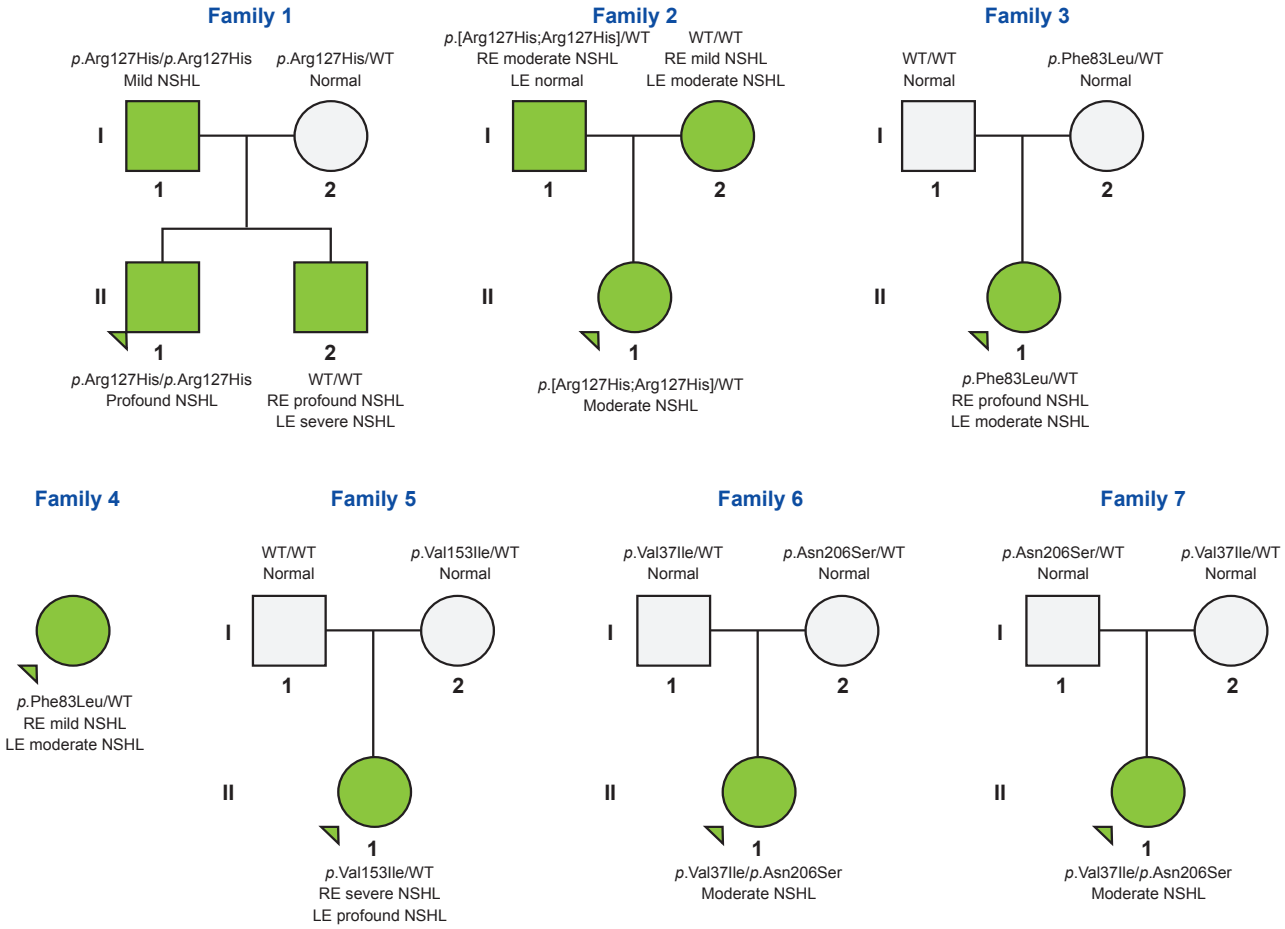

Figure 1 – Genogram of the seven families. Hearing-impaired symptomatic individuals are indicated by green symbols, unaffected patients by light grey symbols. The severity of SNHL considering the $PTA_{0.5,1,2,4kHz}$ is presented: normal hearing corresponds to a PTA < 20 dB, mild SNHL to 21 - 40 dB, moderate SNHL to 41 - 70 dB, severe SNHL to 71 - 95 dB and profound SNHL to PTA > 95 dB. The index case is identified by a green arrow.

WT: wild type; NSHL: non-syndromic hearing loss; RE: right ear; LE: left ear

plained of dizziness or tinnitus, nor reported barotrauma or occupational, recreational, or accidental noise exposure. Some individuals had history of otological disease. Clinical features are summarized in Table 2. Parents from families one, two and four were consanguineous. Also, families one and two were of Romani ethnicity.

The *p*.Phe83Leu variant was present in the heterozygous state in families three and four. Since NS-SNHL is mostly of autosomal recessive inheritance, it is unlikely that this variant is the cause of the condition. Furthermore, in family three, the mother of the index case has the same *GJB2* genotype and normal hearing.

The *p*.Arg127His variant was identified in patients from two different families, both Romani – families one and two − either in heterozygosity or homozygosity or *cis* configuration of two *p*.Arg127His variants. The *cis* configuration was inferred in individuals I-1 and II-1 from family two since the individual II-1 inherited both variants from the male progenitor. The *p*.Arg127His variant does not seem to segregate with the hearing impairment, as the individual II-2 in family one presented with severe to profound NS-SNLH despite the absence of this variant.

The *p*.Val153Ile variant was found in heterozygosity in two individuals from family five. Following the same line of reasoning used for the *p*.Phe83Leu variant, it is unlikely that this variant is the cause of the hearing impairment.

The *p*.Asn206Ser variant was found in compound heterozygosity with the *p*.Val37Ile variant in two individuals from families six and seven, both presenting moderate NS-SNHL bilaterally. Families six and seven are not related, but both came from the same region of southern Portugal. As both variants are classified as pathogenic, this compound heterozygosity might be responsible for the hearing loss in these patients.[14,15]

DISCUSSION

In this study, 360 participants from the G21 cohort were screened for the presence of ten less common *GJB2*

Table 2 – Clinical characteristics of individuals from the SNHL families harbouring the variants *p*.Phe83Leu, *p*.Arg127His, *p*.Val153Ile and *p*.Asn206Ser. The severity of SNHL considering the $PTA_{0.5,1,2,4kHz}$ is presented: normal hearing corresponds to a PTA < 20 dB, mild SNHL to 21 - 40 dB, moderate SNHL to 41 - 70 dB, severe SNHL to 71 - 95 dB and profound SNHL to PTA > 95 dB.

Family	Case	Age (years)	Age at SNHL onset	Hearing impairment	Genotype	Observation
1*,†	I-1	54		Bilateral mild	*p*.Arg127His/*p*.Arg127His	–
	I-2	47	–	Normal audition	*p*.Arg127His/WT	–
	II-1	24	18 months	Bilateral profound	*p*.Arg127His/*p*.Arg127His	Bilateral prosthesis
	II-2	17	16 months	RE profound and LE severe	WT/WT	RE cochlear implant
2*,†	I-1	58	–	RE mixed moderate and LE normal	*p*.[Arg127His;Arg127His]/WT	RE chronic otitis media
	I-2	50	–	RE mild and mixed moderate LE	WT/WT	LE tympanosclerosis
	II-1	20	6 years	Bilateral mixed moderate	*p*.[Arg127His;Arg127His]/WT	Bilateral prosthesis; LE chronic otitis media; RE tympanoplasty; Bilateral myringotomy and tubes at 4 - 5 yo.
3	I-1	56	–	Normal audition	WT/WT	–
	I-2	54	–	Normal audition	*p*.Phe83Leu/WT	–
	II-1	32	Childhood	RE profound and LE moderate	*p*.Phe83Leu/WT	LE prosthesis
4*	–	5	Congenital	RE mild and LE moderate	*p*.Phe83Leu/WT	Bilateral prosthesis
5	I-1	69	–	Normal audition	WT/WT	–
	I-2	64	–	Normal audition	*p*.Val153Ile/WT	–
	II-1	41	1 year	RE severe and LE profound	*p*.Val153Ile/WT	–
6	I-1	34	–	Normal audition	*p*.Val37Ile/WT	–
	I-2	35	–	Normal audition	*p*.Asn206Ser/WT	–
	II-1	3	Congenital	Bilateral moderate	*p*.Val37Ile/p.Asn206Ser	Bilateral prosthesis
7	I-1	50	–	Normal audition	*p*.Asn206Ser/WT	–
	I-2	42	–	Normal audition	*p*.Val37Ile/WT	–
	II-1	7	18 months	Bilateral moderate	*p*.Val37Ile/*p*.Asn206Ser	Bilateral prosthesis

ABG: air-bone gap; RE: right ear; LE: left ear; WT: wild type
* Consanguineous parents; † Romani ethnicity.

variants: *p*.Ile20Met, *p*.Trp24X, *p*.Ile30Val, *p*.Val37Ile, *p*.Phe83Leu, *p*.Val95Met, *p*.Arg127His, *p*.Arg143Gln, *p*.Val153Ile, *p*.Arg184Trp and *p*.Asn206Ser. These variants were selected through the evaluation of a cohort of 147 SNHL patients who had been screened for *GJB2* variants; every detected variant other than *p*.Gly12Valfs*2 (c.35delG) and *p*.Met34Thr was included in our set of less common variants. Even though each less common variant was present in few patients, together they comprised approximately 12% of the SNHL patients included in this study, denoting the relevance in approaching them. An important tool for the classification of the variants regarding pathogenicity is the assessment of their frequency in a healthy population. To our knowledge, this is the first study to estimate the frequency of these variants in a Portuguese community sample.

Four out of ten less common variants were found in individuals from the G21 cohort: *p*.Phe83Leu, *p*.Arg127His, *p*.Val153Ile and *p*.Asn206Ser. All four are missense variants and are classified as benign/likely benign,[16] benign/likely benign/uncertain significance,[17] likely benign,[18] and pathogenic,[14] respectively. Differences between the allelic frequencies of these variants in the G21 sample and the SNHL group were not statistically significant - a finding which was expected for *p*.Phe83Leu, *p*.Arg127His and *p*.Val153Ile, considering their tendentiously benign classifications, but not for *p*.Asn206Ser, whose pathogenicity would be better described by a higher frequency in the SNHL group compared to the G21 sample. For the *p*.Arg127His variant, however, a right deviation of the CI of the SNHL group was observed, suggesting a higher frequency in this group. An explanation can be the fact that the SNHL group also included Romani people, while the G21 group included only participants with Portuguese ancestry. In fact, both index cases identified with the *p*.Arg127His variant were Romani. Similarly, in another Portuguese study consisting of a report on three Portuguese families carrying this variant, two of the families were also Romani.[19] Romani people have Indian ancestry and this variant was found at a high frequency in Indian individuals.[20] Also, in a study with Slovak Romani hearing impaired people, *p*.Arg127His was the most common *GJB2* variant, occurring in 19.4% of the chromosomes screened.[21] So, the inclusion of Romani people may explain the trend for a higher allelic frequency in the SNHL group.

A key point to mention is that the highest estimate for the allelic frequencies of *p*.Arg127His and *p*.Val153Ile variants in the G21 sample is higher than 1%, allowing for these variants to comply with polymorphism criteria. The role of the *p*.Arg127His variant in SNHL is contentious as functional studies are inconsistent.[22–25] Evidence of its possible non-pathogenic nature relies on its common occurrence in the Indian population and the similar frequencies between hearing and non-hearing subjects found in France.[7,20] Moreover, the *p*.Arg127His variant has been detected in normal hearing subjects both at homozygous state[7,26] and in compound heterozygosity with the *p*.Gly12Valfs*2 mutation.[27] Nevertheless, two studies report significantly higher frequencies in the patient group compared to the control group, one from Tibet[28] and the other from India.[29] In the Indian study, compound heterozygosity involving the *p*.Arg127His variant was identified in hearing-impaired individuals but not among control individuals.[29] In this regard, some studies suggest that genotypes combining *p*.Arg127His variant with other pathogenic variants could lead to hearing loss by having phenotypic expression modulated by environmental factors or modifier genes.[7,19,29] Regarding the *p*.Val153Ile variant, the possibility of being pathogenic has been previously proposed.[30–33] However, subsequent studies contradicted this hypothesis by reporting its high occurrence in normal hearing populations,[20,34] and describing its presence in normal hearing subjects both at the homozygous state[20,35] and in compound heterozygosity with the *p*.Gly12Valfs*2 (35delG) mutation.[27,34] Finally, in vitro expression studies in transfected HeLa cells demonstrated that the mutated *p*.Val153Ile protein was correctly synthesized and targeted to the plasma membrane and its function was not altered.[35] It is noteworthy that some studies do not rule out the possible role of the *p*.Val153Ile variant as a modifier of the final phenotype in the presence of other mutations in genes involved in hearing function.[30,32]

The *p*.Phe83Leu variant was first described in 1998[36] and since then, it has been reported by several authors as a polymorphism, as similar frequencies in affected individuals and controls have been observed - likewise in the present study - and, in familial studies, it was not segregating with SNHL.[37-42] Furthermore, functional evidence supports its non-pathogenic nature.[43]

In this study, the *p*.Ans206Ser variant was detected in two index cases and, interestingly, both presented compound heterozygosity with the pathogenic variant *p*.Val37Ile.[44] Also, both children had bilateral moderate NS-SNHL ($PTA_{0.5,1,2,4kHz}$ in the range of 41 - 70 dB). Compound heterozygous genotypes with *p*.Asn206Ser have received special attention in previous studies, as they have been associated with less severe audiological characteristics.[45] In fact, the genotype *p*.Val37Ile/*p*.Ans206Ser was previously identified in a patient with congenital bilateral mild NS-SNHL ($PTA_{0.5,1,2kHz}$ of 24 dB).[33] Furthermore, the genotype *p*.Gly12Valfs*2/*p*.Ans206Ser was associated with bilateral moderate SNHL in three patients ($PTA_{0.5,1,2,3,4,6,8kHz}$ in the range of 41 - 70 dB[7] ; $PTA_{0.5,1,2kHz}$ in the range of 41 - 55 dB[46] and $PTA_{0.5,1,2,4kHz}$ of 65 dB)[47] and unilateral mild SNHL in another ($PTA_{0.5,1,2kHz}$ in the range of 21 - 40 dB).[46] These findings led the authors to speculate that the *p*.Asn2016Ser variant may not severely compromise the gap junctional communication system in the inner ear,[46] which was later corroborated by functional studies - some even revealing that the permeability to anionic fluorescent tracers was maintained, but the permeability to larger molecules was compromised.[31,48,49]

The comparison between the allelic frequencies of the G21 group and the GnomAD European sample revealed statistically significant differences for the *p*.Val153Ile and *p*.Asn206Ser variants, suggesting that these variants might

be more frequent in the Portuguese population. In fact, our allelic rate for *p*.Val153Ile (0.97%) is higher than the frequencies described for Italy (0.49%)[35] and France (0.38%),[7] but lower than that for the Czech Republic (1.92%).[34] Variants *p*.Phe83Leu and *p*.Arg127His variant did not show statistically significant differences, even though our G21 allelic frequencies (0.14% for *p*.Phe83Leu and 0.28% for *p*.Arg127His) were lower than frequencies described for France (0.28% and 0.66%, respectively).[7]

Another important consideration of our study is the detection of the Arg127His variant in a *cis* configuration (family 2, Fig.1) since it has never been described in previous research, as far as we know.

The variants *p*.Trp24Ter, *p*.Val37Ile and *p*.Arg184Trp, classified as pathogenic,[15,50,51] and the variants *p*.Val95Met and *p*.Arg143Gln, classified as pathogenic/likely pathogenic,[52,53] were not identified in individuals from the G21 cohort, which is consistent with their classifications. The *p*.Ile30Val, a variant of uncertain significance,[54] was not detected either. The differences in allelic frequencies between the G21 and the SNHL groups for the *p*.Val37Ile and the *p*.Val95Met variants were statistically significant, strengthening the hypothesis of their pathogenic role in SNHL. No statistically significant differences were observed for the other variants.

CONCLUSION

The present study is the first report of the frequency of the less common *GJB2* variants in a Portuguese sample. The *p*.Phe83Leu, *p*.Arg127His, *p*.Val153Ile and *p*.Asn206Ser variants were identified in G21 participants, in heterozygosity, with the allelic frequencies of 0.14%, 0.28%, 0.97% and 0.14%, respectively. The *p*.Trp24Ter, *p*.Ile30Val, *p*.Val37Ile, *p*.Val95Met, *p*.Arg143Gln and *p*.Arg184Trp variants were not found in the G21 group. Our estimate of the allelic frequencies allows for the *p*.Arg127His and *p*.Val153Ile variants to comply with polymorphism criteria. Furthermore, our comparison of the allelic frequencies of the variants between G21 and the patients' group strengthens the hypothesis of *p*.Val37Ile and the *p*.Val95Met variants having a pathogenic role in NS-SNHL. Moreover, these results raise the hypothesis that *p*.Val153Ile and *p*.Asn206Ser variants are more frequent in the northern Portuguese population than in the general European population. Estimating the carrier rates of the variants in a healthy population is an important tool for the classification of their pathogenicity. The clarification of the pathogenic role of each variant is of paramount importance in familial genetic counselling.

AUTHORS CONTRIBUTION

CSR: Data analysis and interpretation; draft of the manuscript.

ACS, HB: Cohort design and management; critical review of the paper.

SF: Design of the work; data interpretation; critical review of the paper.

CPM: Design of the work; data interpretation; critical review of the paper; approval of the final version.

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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 2013 Helsinki Declaration of the World Medical Association.

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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REFERENCES

1. Morton CC, Nance WE. Newborn hearing screening - a silent revolution. N Engl J Med. 2006;354:2151-64.
2. Lameiras AR, Gonçalves AC, Santos R, O'Neill A, Reis LR dos, Matos TD, et al. The controversial p.Met34Thr variant in *GJB2* gene: two siblings, one genotype, two phenotypes. Int J Pediatr Otorhinolaryngol. 2015;79:1316-9.
3. Nogueira C, Coutinho M, Pereira C, Tessa A, Santorelli FM, Vilarinho L. Molecular investigation of pediatric portuguese patients with sensorineural hearing loss. Genet Res Int. 2011;2011:587602.
4. Kenneson A, Van Naarden Braun K, Boyle C. *GJB2* (connexin 26) variants and nonsyndromic sensorineural hearing loss: a HuGE review. Genet Med. 2002;4:258-74.
5. Stenson PD, Mort M, Ball EV, Howells K, Phillips AD, Thomas NS, et al. The Human Gene Mutation Database: 2008 update. Genome Med. 2009;1:13.
6. Pandya A, Arnos KS, Xia XJ, Welch KO, Blanton SH, Friedman TB, et al. Frequency and distribution of *GJB2* (connexin 26) and GJB6 (connexin 30) mutations in a large North American repository of deaf probands. Genet Med. 2003;5:295-303.
7. Roux AF, Pallares-Ruiz N, Vielle A, Faugère V, Templin C, Leprevost D,

et al. Molecular epidemiology of DFNB1 deafness in France. BMC Med Genet. 2004;5:5.
8. Dalamón V, Lotersztein V, Béhèran A, Lipovsek M, Diamante F, Pallares N, et al. *GJB2* and GJB6 genes: molecular study and identification of novel *GJB2* mutations in the hearing-impaired Argentinean population. Audiol Neurootol. 2010;15:194-202.
9. Matos TD, Simões-Teixeira H, Caria H, Gonçalves AC, Chora J, Correia M do C, et al. Spectrum and frequency of *GJB2* mutations in a cohort of 264 Portuguese nonsyndromic sensorineural hearing loss patients. Int J Audiol. 2013;52:466-71.
10. Kelsell DP, Dunlop J, Stevens HP, Lench NJ, Liang JN, Parry G, et al. Connexin 26 mutations in hereditary non-syndromic sensorineural deafness. Nature. 1997;387:80-3.
11. Dória M, Neto AP, Santos AC, Barros H, Fernandes S, Moura CP. Prevalence of 35delG and Met34Thr *GJB2* variants in Portuguese samples. Int J Pediatr Otorhinolaryngol. 2015;79:2187-90.
12. Karczewski KJ, Francioli LC, Tiao G, Cummings BB, Alföldi J, Wang Q, et al. Variation across 141,456 human exomes and genomes reveals the spectrum of loss-of-function intolerance across human protein-coding genes. BioRxiv. 2019:531210.
13. Agresti A, Coull BA. Approximate is Better than "Exact" for Iinterval estimation of binomial proportions. Am Stat. 1998;52:119-26.
14. ClinVar. Bethesda: U.S. National Library of Medicine, National Center for Biotechnology Information; c2019-12. NM_004004.6(GJB2):c.617A>G (p.Asn206Ser). [accessed 2019 Jan 7]. Available from: https://www.ncbi.nlm.nih.gov/clinvar/variation/44763/.
15. ClinVar. Bethesda: U.S. National Library of Medicine, National Center for Biotechnology Information; c2019-12. NM_004004.6(GJB2):c.109G>A (p.Val37Ile). [accessed 2019 Jan 7]. Available from: https://www.ncbi.nlm.nih.gov/clinvar/variation/17023/.
16. ClinVar. Bethesda: U.S. National Library of Medicine, National Center for Biotechnology Information; c2019-12. NM_004004.6(GJB2):c.249C>G (p.Phe83Leu). [accessed 2019 Jan 7]. Available from: https://www.ncbi.nlm.nih.gov/clinvar/variation/44732/.
17. ClinVar. Bethesda: U.S. National Library of Medicine, National Center for Biotechnology Information; c2019-12. NM_004004.6(GJB2):c.380G>A (p.Arg127His). [accessed 2019 Jan 7]. Available from: https://www.ncbi.nlm.nih.gov/clinvar/variation/44745/.
18. ClinVar. Bethesda: U.S. National Library of Medicine, National Center for Biotechnology Information; c2019-12. NM_004612.4(TGFBR1):c.457G>A (p.Val153Ile). [accessed 2019 Jan 7]. Available from: https://www.ncbi.nlm.nih.gov/clinvar/variation/213868/.
19. Matos TD, Simões-Teixeira H, Caria H, Rosa H, O'Neill A, Fialho G. The controversial p.Arg127His mutation in *GJB2*: report on three Portuguese hearing loss family cases. Genet Test Mol Biomarkers. 2010;14:141-4.
20. RamShankar M, Girirajan S, Dagan O, Ravi Shankar HM, Jalvi R, Rangasayee R, et al. Contribution of connexin26 (*GJB2*) mutations and founder effect to non-syndromic hearing loss in India. J Med Genet. 2003;40:e68.
21. Minárik G, Ferák V, Feráková E, Ficek A, Poláková H, Kádasi L. High frequency of *GJB2* mutation W24X among Slovak Romany (Gypsy) patients with non-syndromic hearing loss (NSHL). Gen Physiol Biophys. 2003;22:549-56.
22. Thönnissen E, Rabionet R, Arbonès ML, Estivill X, Willecke K, Ott T. Human connexin26 (*GJB2*) deafness mutations affect the function of gap junction channels at different levels of protein expression. Hum Genet. 2002;111:190-7.
23. D'Andrea P, Veronesi V, Bicego M, Melchionda S, Zelante L, Di Iorio E, et al. Hearing loss: frequency and functional studies of the most common connexin26 alleles. Biochem Biophys Res Commun. 2002;296:685-91.
24. Wang HL, Chang WT, Li AH, Yeh TH, Wu CY, Chen MS, et al. Functional analysis of connexin-26 mutants associated with hereditary recessive deafness. J Neurochem. 2003;84:735-42.
25. Palmada M, Schmalisch K, Böhmer C, Schug N, Pfister M, Lang F, et al. Loss of function mutations of the *GJB2* gene detected in patients with DFNB1-associated hearing impairment. Neurobiol Dis. 2006;22:112-8.
26. Dahl HH, Tobin SE, Poulakis Z, Rickards FW, Xu X, Gillam L, et al. The contribution of *GJB2* mutations to slight or mild hearing loss in Australian elementary school children. J Med Genet. 2006;43:850-5.
27. Marlin S, Garabédian ÉN, Roger G, Moatti L, Matha N, Lewin P, et al. Connexin 26 gene mutations in congenitally deaf children: pitfalls for genetic counseling. Arch Otolaryngol Head Neck Surg. 2001;127:927-33.
28. Yuan Y, Zhang X, Huang S, Zuo L, Zhang G, Song Y, et al. Common molecular etiologies are rare in nonsyndromic Tibetan Chinese patients with hearing impairment. PLoS ONE. 2012;7:e30720.
29. Adhikary B, Ghosh S, Paul S, Bankura B, Pattanayak AK, Biswas S, et al. Spectrum and frequency of *GJB2*, GJB6 and SLC26A4 gene mutations among nonsyndromic hearing loss patients in eastern part of India. Gene. 2015;573:239-45.
30. Wu BL, Lindeman N, Lip V, Adams A, Amato RS, Cox G, et al. Effectiveness of sequencing connexin 26 (*GJB2*) in cases of familial or sporadic childhood deafness referred for molecular diagnostic testing. Genet Med. 2002;4:279-88.
31. Meşe G, Londin E, Mui R, Brink PR, White TW. Altered gating properties of functional Cx26 mutants associated with recessive non-syndromic hearing loss. Hum Genet. 2004;115:191-9.
32. Dalamón V, Béhèran A, Diamante F, Pallares N, Diamante V, Elgoyhen AB. Prevalence of *GJB2* mutations and the del(GJB6-D13S1830) in Argentinean non-syndromic deaf patients. Hear Res. 2005;207:43-9.
33. Snoeckx RL, Huygen PL, Feldmann D, Marlin S, Denoyelle F, Waligora J, et al. *GJB2* mutations and degree of hearing loss: a multicenter study. Am J Hum Genet. 2005;77:945-57.
34. Seeman P, Malíková M, Rasková D, Bendová O, Groh D, Kubálková M, et al. Spectrum and frequencies of mutations in the *GJB2* (Cx26) gene among 156 Czech patients with pre-lingual deafness. Clin Genet. 2004;66:152-7.
35. Guerci VI, Grasso DL, Morgutti M, Amoroso A, D'Andrea P, Bicego M, et al. Connexin 26 gene: defining the role of the V1531 mutation. Hear Balance Commun. 2007;5:200-6.
36. Scott DA, Kraft ML, Carmi R, Ramesh A, Elbedour K, Yairi Y, et al. Identification of mutations in the connexin 26 gene that cause autosomal recessive nonsyndromic hearing loss. Hum Mutat. 1998;11:387-94.
37. Tang HY, Fang P, Ward PA, Schmitt E, Darilek S, Manolidis S, et al. DNA sequence analysis of *GJB2*, encoding connexin 26: observations from a population of hearing impaired cases and variable carrier rates, complex genotypes, and ethnic stratification of alleles among controls. Am J Med Genet A. 2006;140:2401-15.
38. Cheng X, Li L, Brashears S, Morlet T, Ng SS, Berlin C, et al. Connexin 26 variants and auditory neuropathy/dys-synchrony among children in schools for the deaf. Am J Med Genet A. 2005;139:13-8.
39. Santos RL, Aulchenko YS, Huygen PL, van der Donk KP, de Wijs IJ, Kemperman MH, et al. Hearing impairment in Dutch patients with connexin 26 (*GJB2*) and connexin 30 (GJB6) mutations. Int J Pediatr Otorhinolaryngol. 2005;69:165-74.
40. Heathcote K, Syrris P, Carter ND, Patton MA. A connexin 26 mutation causes a syndrome of sensorineural hearing loss and palmoplantar hyperkeratosis (MIM 148350). J Med Genet. 2000;37:50-1.
41. Rabionet R, Zelante L, López-Bigas N, D'Agruma L, Melchionda S, Restagno G, et al. Molecular basis of childhood deafness resulting from mutations in the *GJB2* (connexin 26) gene. Hum Genet. 2000;106:40-4.
42. Picciotti PM, Pietrobono R, Neri G, Paludetti G, Fetoni AR, Cianfrone F, et al. Correlation between *GJB2* mutations and audiological deficits: personal experience. Eur Arch Otorhinolaryngol. 2009;266:489-94.
43. Bruzzone R, Veronesi V, Gomès D, Bicego M, Duval N, Marlin S, et al. Loss-of-function and residual channel activity of connexin26 mutations associated with non-syndromic deafness. FEBS Lett. 2003;533:79-88.
44. Shen J, Oza AM, Del Castillo I, Duzkale H, Matsunaga T, Pandya A, et al. Consensus interpretation of the p.Met34Thr and p.Val37Ile variants in *GJB2* by the ClinGen Hearing Loss Expert Panel. Genet Med. 2019;21:2442-52.
45. Marlin S, Feldmann D, Blons H, Loundon N, Rouillon I, Albert S, et al. *GJB2* and GJB6 mutations: genotypic and phenotypic correlations in a large cohort of hearing-impaired patients. Arch Otolaryngol Head Neck Surg. 2005;131:481-7.
46. Kenna MA, Wu BL, Cotanche DA, Korf BR, Rehm HL. Connexin 26 studies in patients with sensorineural hearing loss. Arch Otolaryngol Head Neck Surg. 2001;127:1037-42.
47. Cryns K, Orzan E, Murgia A, Huygen PL, Moreno F, del Castillo I, et al. A genotype-phenotype correlation for *GJB2* (connexin 26) deafness. J Med Genet. 2004;41:147-54.
48. Xu J, Nicholson BJ. The role of connexins in ear and skin physiology - functional insights from disease-associated mutations. Biochim Biophys Acta. 2013;1828:167-78.
49. Ambrosi C, Walker AE, Depriest AD, Cone AC, Lu C, Badger J, et al. Analysis of trafficking, stability and function of human connexin 26 gap junction channels with deafness-causing mutations in the fourth transmembrane helix. PLoS ONE. 2013;8:e70916.
50. ClinVar. Bethesda: U.S. National Library of Medicine, National Center for Biotechnology Information; c2019-12. NM_004004.6(GJB2):c.71G>A (p.Trp24Ter). [accessed 2019 Jan 7]. Available from: https://www.ncbi.

nlm.nih.gov/clinvar/variation/17002/.
51. ClinVar. Bethesda: U.S. National Library of Medicine, National Center for Biotechnology Information; c2019-12. NM_004004.6(GJB2):c.550C>T (p.Arg184Trp). [accessed 2019 Jan 7]. Available from: https://www.ncbi.nlm.nih.gov/clinvar/variation/560669/.
52. ClinVar. Bethesda: U.S. National Library of Medicine, National Center for Biotechnology Information; c2019-12. NM_004004.6(GJB2):c.283G>A (p.Val95Met). [accessed 2019 Jan 7]. Available from: https://www.ncbi.nlm.nih.gov/clinvar/variation/44735/.
53. ClinVar. Bethesda: U.S. National Library of Medicine, National Center for Biotechnology Information; c2019-12. NM_004004.6(GJB2):c.428G>A (p.Arg143Gln). [accessed 2019 Jan 7]. Available from: https://www.ncbi.nlm.nih.gov/clinvar/variation/17017/.
54. ClinVar. Bethesda: U.S. National Library of Medicine, National Center for Biotechnology Information; c2019-12. NM_000503.6(EYA1):c.88A>G (p.Ile30Val). [accessed 2019 Jan 7]. Available from: https://www.ncbi.nlm.nih.gov/clinvar/variation/517593/.

Health-Related Quality of Life in Survivors of Severe COVID-19 of a University Hospital in Northern Portugal

Qualidade de Vida dos Sobreviventes da COVID-19 Grave de um Hospital Universitário no Norte de Portugal

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ABSTRACT

Introduction: Long-term health impairments are often experienced among survivors of critical illness, which may have a negative impact on their quality of life. The aim of this study was to characterize COVID-19 survivors of critical illness and to evaluate health-related quality of life and disability following hospital discharge.

Material and Methods: This is a retrospective case-series study that included COVID-19 survivors admitted to the Intensive Care Medicine Department of a University Hospital. Follow-up evaluation was performed between the 30th and the 90th day after discharge. Quality of life was explored using the five-level version of the EQ-5D instrument (EQ-5D-5L) and functionality using the 12-question World Health Organization Disability Assessment Schedule 2.0 (WHODAS 2.0).

Results: Forty-five survivors were enrolled, 28 (62.2%) men, median age 63.0 years. The EQ-5D-5L questionnaire showed moderate to extreme problems in some dimension in 29 patients (64.4%): mobility in six (13.3%), self-care in seven (13.3%), usual activities in 23 (51.1%), pain/discomfort in 14 (31.1%) and anxiety/depression in 17 (37.8%). When using the 12-question WHODAS 2.0 questionnaire, moderate to extreme disability was reported in some question in 37 patients (82.2%): 19 (42.2%) in standing for long periods, 18 (40.0%) in long-distance walking; 14 (31.1%) on taking care of household responsibilities and 17 (37.8%) in their day-to-day work; 23 (51.1%) felt emotionally affected by their health problems.

Discussion: Based on COVID-19 survivors-reported outcomes after critical illness, mobility, pain/discomfort, and anxiety/depression were the main problems that persisted one to three months after hospital discharge.

Conclusion: An organized follow-up structure is crucial to improve health-related quality of life in critical COVID-19 survivors.

Keywords: COVID-19; Critical Care; Follow-up Studies; Portugal; Quality of Life; Survivors

RESUMO

Introdução: Os sobreviventes de doença crítica apresentam frequentemente sequelas a longo prazo. O objetivo deste estudo foi caracterizar os sobreviventes da COVID-19 grave e avaliar a qualidade de vida após a alta hospitalar.

Material e Métodos: Série de casos que inclui sobreviventes COVID-19 admitidos no Serviço de Medicina Intensiva de um Hospital Universitário. A consulta de seguimento foi realizada entre o 30º e o 90º dia após alta hospitalar. A qualidade de vida foi avaliada através do questionário EQ-5D com cinco níveis (EQ-5D-5L) e a funcionalidade através do instrumento *World Health Organization Disability Assessment Schedule* 2.0 (WHODAS 2.0) de 12 questões.

Resultados: Foram incluídos 45 sobreviventes, 28 homens (62,2%), idade mediana de 63,0 anos. No questionário EQ-5D-5L 29 sobreviventes (64,4%) mostraram problemas moderados a extremos em alguma dimensão: seis (13,3%) na mobilidade, sete (13,3%) nos cuidados pessoais, 23 (51,1%) nas atividades habituais, 14 (31,1%) na dor/desconforto e 17 (37,8%) na ansiedade/depressão. No WHODAS 2.0 37 sobreviventes (82,2%) revelaram alterações funcionais moderadas a extremas em alguma questão: 19 (42,2%) em permanecer de pé por longos períodos, 18 (40,0%) em percorrer longas distâncias, 14 (31,1%) em cuidar das responsabilidades domésticas e 17 (37,8%) no dia-a-dia no trabalho; 23 (51,1%) mostraram-se emocionalmente afetados pelos seus problemas de saúde.

Discussão: A avaliação dos sobreviventes COVID-19 após a doença crítica demonstra que a mobilidade, a dor/desconforto e a ansiedade/depressão são os principais problemas que persistem um a três meses após a alta hospitalar.

Conclusão: O acompanhamento estruturado após alta poderá ter impacto significativo na qualidade de vida destes doentes.

Palavras-chave: COVID-19; Medicina Intensiva; Portugal; Qualidade de Vida; Seguimentos; Sobreviventes

INTRODUCTION

On the 2nd of March of 2020, the first case in Portugal of infection with the novel severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) was diagnosed.[1] This challenging disease with a daily stunning speed of infection led to abrupt adjustments in hospital and healthcare teams, with profound consequences to the physical and mental health of all those involved: professionals, patients and families.[2,3] A recent meta-analysis reported that approximately 20% of COVID-19 hospitalized patients required admission to Intensive Care Medicine.[4]

Long-term impairment in physical, cognitive and mental health after critical illness are often experienced among survivors and their families, which is known as post-intensive care syndrome (PICS).[5] One year after critical illness, 60% of survivors have one or more PICS-related problems.[6] Moreover, moderate or severe disability six months after critical illness is present in 25% of survivors and it is associated with reduced health-related quality of life.[7] The pressing question remains understanding what the outcomes of COVID-19 patients are after discharge from Intensive Care and what are the implications of PICS.[8] The largest clinical follow-up study published about COVID-19 patients

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reported that the severity of illness was a risk factor for psychological symptoms, mobility problems, persistent pain/discomfort, and anxiety/depression in survivors.[9]

The aim of this study was to characterize survivors of COVID-19 critical illness and to evaluate health-related quality of life and disability following hospital discharge.

MATERIAL AND METHODS

Study design and participants

This retrospective case-series study included all COVID-19 survivors admitted to the Intensive Care Medicine Department of Centro Hospitalar Universitário São João in Porto, Portugal, with an effective hospital discharge until the 15th of July of 2020. The eligible adult survivors were those with an intensive care length of stay lasting longer than 24 hours.

In this Intensive Care Medicine Department there is a follow-up clinic dedicated to the assessment of patients after critical illness which includes intensivists and an intensive care trained nurse specifically dedicated to contacting survivors by telephone and to apply disability scales as a triage method before medical evaluation. The evaluation period of survivors includesthe period between the date of hospital discharge and the date of clinical telephone evaluation.

The study was approved by the Ethics Committee of Centro Hospitalar Universitário São João (CE 376/2020) and all the included patients gave verbal informed consent at the time of contact.

Data collection

Demographic, clinical, laboratory and treatment data were extracted from the hospital electronic information systems. All patients had laboratory confirmation of SARS-CoV-2 infection by real-time PCR methods.

Follow-up evaluation of survivors was performed over the telephone by the intensive care nurse of the Intensive Care follow-up team, between the 30th and the 90th day after hospital discharge, following the specific requirements of each scale evaluated in this study. Answers were provided by the patient, except in three cases in which the family did it.

Health-related quality of life

Health-related quality of life was assessed with the EuroQol five-dimension five-level questionnaire (EQ-5D-5L). This is a descriptive self-evaluation that assesses five dimensions: mobility, self-care (hygiene and dressing), usual activities (work, study, housework, family and leisure activities), pain/discomfort and anxiety/depression. Each dimension has five levels of disability: no problems, slight problems, moderate problems, severe problems and unable to or extreme problems, classified between 1 and 5.[10] The visual analogue scale (EQ-VAS) is a quantitative measure of health outcomes that reflects the self-rated health of patients, where the endpoints are labelled between “the worst health you can imagine” (zero points) and “the best health you can imagine” (100 points).[10] The results will be presented as disability degree for each dimension. We applied the validated EQ-5D-5L Portuguese version.[11]

Functionality and disability

In order to complement the evaluation of the impact of critical illness on global functionality and disability the 12-question World Health Organization Disability Assessment Schedule 2.0 (WHODAS 2.0) was applied. It covers six domains of functioning, each one based on two questions with the intention of recognizing functional impairments in the last 30 days: cognition (understanding and communication), mobility (moving and getting around), self-care (hygiene, dressing, eating and staying alone), getting along (interacting with other people), life activities (domestic responsibilities, leisure, work and school) and participation (joining community activities). Each question was scored from 1 (no difficulty) to 5 (extreme difficulty or cannot do) with the possibility of answering “not applicable” (N/A) if the person did not have the opportunity to complete the task in the last 30 days.[12] The results will be presented as disability degree in each question. We applied the validated Portuguese version of 12-question WHODAS 2.0.[13]

Statistical analysis

Statistical analysis was carried out using SPSS software (version 23.0). Continuous variables were presented as median (interquartile range) and categorical variables as frequency rates (percentages).

The answers to the EQ-5D-5L and WHODAS 2.0 questionnaires were dichotomized into no or mild problems/disability (score 1 or 2) and moderate to extreme problems/disability (score 3, 4 or 5). For the comparison of disability degree according to whether invasive mechanical ventilation (IMV) was used or not, and according to the period in which the follow-up assessment was performed (between day 30 and 44 or between day 45 and 90), we used the Mann-Whitney U test. *P*-values < 0.05 were considered significant.

RESULTS

Population characterization

A total of 93 adult critically ill patients infected with SARS-CoV-2 were admitted to the Intensive Care Medicine Department of Centro Hospitalar Universitário São João from the 11th of March to the 10th June 2020. Among the 86 patients that stayed in the Intensive Care Medicine Department for more than 24 hours, 46 (53.5%) were already home by the 15th of July and were eligible for this study, 23 died during hospital stay (26.7%) and 17 (19.8%) were still hospitalized (Fig. 1). One patient of the 46 survivors refused to participate in this study.

Demographic and clinical characteristics of survivors are detailed in Table 1. All enrolled patients were admitted with the diagnosis of SARS-CoV-2 pneumonia. Forty-one (91.1%) were supported with some type of mechanical ventilation. High flow nasal cannula (HFNC) was used in 20

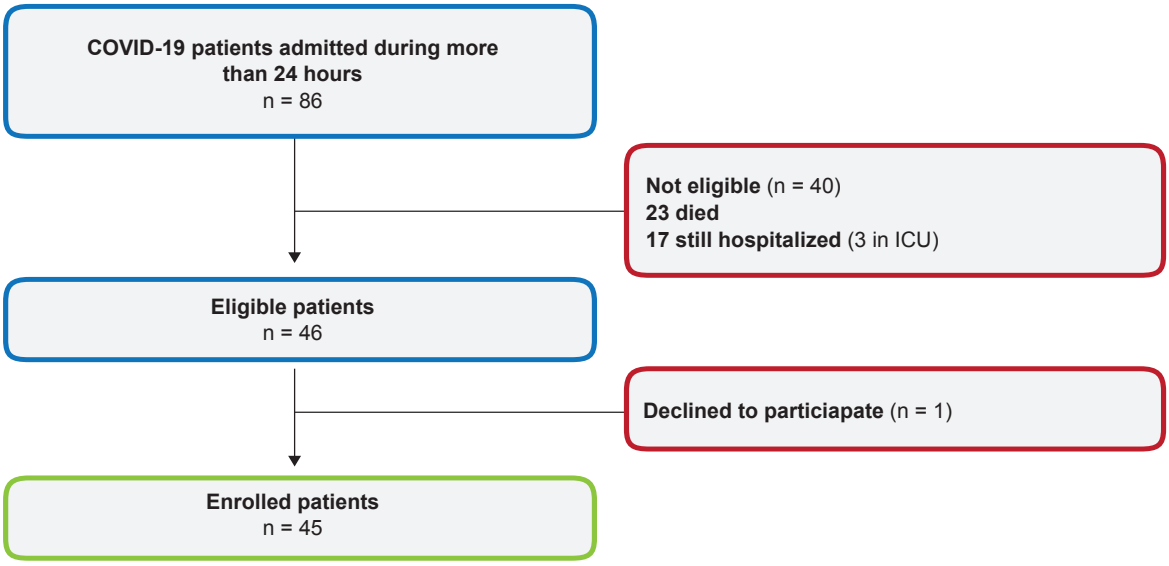

Figure 1 – Flow diagram of studied participants

Table 1 – Demographic and clinical characteristics of 45 COVID-19 survivors after critical illness

Demographic and clinical characteristics	All patients (n = 45)	IMV patients (n = 31)	No-IMV patients (n = 14)	*p* value
Age, years (IQR)	63 (55 - 73)	63 (49 - 73)	62 (59 - 74)	0.75
Male, n (%)	28 (62%)	20 (65%)	8 (57%)	0.64
First symptom to hospital admission, days (IQR)	6 (3 - 8)	6 (3 - 9)	4 (3 - 7)	0.48
Hypertension, n (%)	29 (64%)	22 (71%)	7 (50%)	0.19
Obesity, n (%)	18 (40%)	12 (39%)	6 (43%)	0.79
History of anxiety/depression, n (%)	16 (36%)	10 (32%)	6 (43%)	0.52
Known respiratory disease, n (%)	8 (18%)	5 (16%)	3 (21%)	0.69
Charlson Comorbidity Index, (IQR)	3 (0 - 4)	2 (0 - 4)	3 (2 - 4)	0.18
SAPS II Score (IQR)	36 (25 - 50)	36 (25 - 57)	30 (19 - 41)	0.14
APACHE II Score (IQR)	16 (12 - 22)	17 (13 - 23)	13 (10 - 17)	0.04
ICU length stay, days (IQR)	18 (6 - 25)	21 (15 - 33)	4 (3 - 6)	< 0.001
Hospital length of stay, days (IQR)	31 (15 - 38)	34 (23 - 42)	17 (15 - 27)	0.007
Mechanical ventilation, n (%) Invasive ventilation, n (%) High flow nasal cannula, n (%) Conventional non-invasive ventilation, n (%)	41 (91%) 31 (69%) 20 (44%) 14 (31%)	-	- - 7 (50%) 4 (29%)	-
ECMO, n (%)	6 (13%)	5 (16%)	1 (7%)	-
Continuous renal replacement therapy, n (%)	2 (4%)	2 (6%)	0	-

Results are expressed as n (%) or median (25th - 75th percentiles). IMV: invasive mechanical ventilation; SAPS Score: Simplified Acute Physiology Score II; APACHE II Score: Acute Physiology And Chronic Health Evaluation II; ICU: Intensive Care Unit; ECMO: extracorporeal membrane oxygenation.

patients (44.4%) for a median of 2.0 days (1.3-3.8) and 14 patients (31.1%) were supported with conventional non-invasive mechanical ventilation (NIV) for a median time of 1.0 days (1.0-2.0). Endotracheal intubation and IMV were performed in 31 (68.9%) and maintained for a median period of 18.0 days (11.0-26.0). These patients had a higher APACHE score and a longer ICU and hospital length of stay. Veno-venous Extracorporeal Membrane Oxygenation (ECMO) support was performed in 6 (13.3%), with a median duration of 15.0 days (11.0-18.3). Among 31 patients who underwent deep sedation, fentanyl perfusion (150 (100-200) mcg/h) was used in all of them for a median period of 14 days (8-22) and midazolam infusion (4 (2-6) mg/h) was used in 20 (44.4%) for a median period of eight (4-15) days. Dexmedetomidine was used in 27 patients (60.0%), mainly during the weaning process (96%), representing a sedative/anxiolysis strategy in 85% of patients with IMV, trying to avoid or control the expression of delirium in patients subjected to prolonged deep sedation. Delirium was described in eight patients (17.8%).

Quality of life and disability outcomes

Median time from discharge to follow-up assessment was 55.0 days (42.0-64.0).

Moderate to extreme problems (level ≥ 3) in some dimension of the EQ-5D-5L questionnaire were described in 29 patients (64.4%). The representation of moderate to extreme problems regarding the five dimensions was the following: mobility in six patients (13.3%), self-care in seven patients (13.3%), usual activities in 23 patients (51.1%), pain/discomfort in 14 patients (31.1%) and anxiety/depression in 17 (37.8%). The median EQ-VAS score was 75.0 (60.0 - 90.0).

In the 12-question WHODAS 2.0, 38 survivors (84.4%) reported moderate to extreme functionality impairments in at least one question. Moderate to extreme disability were mostly reported in the following questions: 19 (42.2%) in standing for long periods, 18 (40.0%) in walking a long distance, 17 (37.8%) in their day-to-day work/school responsibilities, 14 (31.1%) in taking care of their household responsibilities, 12 (26.7%) in joining community activities. Twenty-three (51.1%) assumed that they felt emotionally affected by their health problems and 12 of these 23 (52.2%) had no previous anxiety/depression disorders.

Moderate to extreme disability according to whether IMV was used or not, and according to the period in which the follow-up assessment was performed is represented in Table 2. Invasive mechanically ventilated patients reported significantly higher levels of disability in 3 questions of the WHODAS 2.0 questionnaire: standing for long periods (p = 0.04), walking a long distance (p = 0.02) and day-to-day work responsibilities (p = 0.02). There was no association between moderate to extreme disability and the period in which the follow-up assessment was performed.

Of the 19 survivors with an active professional life before hospital admission (42.2%), 15 (78.9%) were still on sick leave and only four (21.1%) had returned to their regular professional activities.

DISCUSSION

In the current case-series study of survivors of COVID-19 critical illness, performed one to three months after discharge, the incidence of moderate to extreme problems in health-related quality of life, assessed by the EQ-5D-5L instrument, was 64% and moderate to extreme disability, evaluated by the WHODAS 2.0 questionnaire, was observed in 84%.

In the scientific literature, one or more PICS related problems are described in 60% of critical illness survivors one year after intensive care admission.[6] Hodgson *et al* described moderate or severe disability six months after critical illness in 25% of survivors and its association with reduced health-related quality of life. They also found that prior history of anxiety/depression and a longer duration of mechanical ventilation were predictors of disability.[7]

The population of critical COVID-19 patients may be particularly prone to develop PICS. Firstly, because risk factors for developing PICS are part of the typical clinical profile of the COVID-19 critical patient.[8] In fact, out of 45 survivors, 62.2% were male, median age was 63.0 years and comorbidities were present in 86.7% of critical survivors, the most prevalent being hypertension (64.4%), followed by obesity (40.0%), anxiety/depression (35.6%) and previous pulmonary disorder (17.8%). Secondly, because median Intensive Care and hospital length of stay are usually long – respectively, 18.0 (6.0 - 25.0) and 31.0 days (14.5 - 37.5) in this population – and prolonged bed rest and extended hospital stay contribute to muscular weakness that is associated with substantial impairments in physical function and health-related quality of life that often persist beyond 24 months after critical illness.[14] Thirdly, because these patients often need prolonged deep sedation[15] and we also observed an unusually high sedation requirements in a large proportion of COVID-19 patients in our clinical practice, which could explain the significant use of midazolam perfusion (44.4%).

In the EQ-5D-5L questionnaire, applied 30 and 90 days after hospital discharge, the most affected dimension was usual activities (51.1% describing moderate to extreme problems), followed by anxiety/depression (37.8% with moderate to extreme problems) and pain/discomfort (31.1% with moderate to extreme problems). These findings are consistent with the results of a recent work from Belfast that highlighted a significant level of functional and psychological morbidity in COVID-19 patients post-intensive care admission where 61% had moderate to severe problems participating in previous activities, 45.2% had at least moderate impairment of mobility and 35.5% described at least moderate symptoms of anxiety/depression at the time of follow-up.[16]

Additionally, in the present study, the 12-question WHODAS 2.0 questionnaire showed that mobility, life activities and participation were the most affected domains: 42% with moderate to extreme difficulty in standing for long periods, 40% in walking a long distance, 37% in day-to-day work/school responsibilities, 31% in joining community activities and 51% emotionally affected by their health problems. The largest clinical follow-up study published with COVID-19 adult patients so far reported that 86% of patients supported with HFNC, NIV or IMV presented at least one symptom six months after symptom onset with an important impact of the critical disease in mobility and physical status: 81% presenting fatigue or muscle weakness and 29% with a distance walked in 6-min that was below the lower limit of the normal range.[9] In fact, we also found that IMV patients reported significantly higher levels of disability in the two questions of the WHODAS 2.0 questionnaire concerning mobility: standing for long periods (p = 0.04) and walking a long distance (p = 0.02).

Psychological impairments were also significant. This can be intrinsically associated with the impact of the pandemic on social isolation and less cognitive stimulation which may exacerbate symptoms of anxiety/depression.[17] An evaluation of self-reported clinical sequelae after hospital discharge of COVID-19 hospitalized patients from

Table 2 – Moderate to extreme disability in EQ-5D-5L and WHODAS 2.0

Parameter	Total (n = 45)	IMV (n = 31)	No IMV (n = 14)	*p* value	Follow-up between 30-44 days (n = 13)	Follow-up between 45-90 days (n = 32)	*p* value
EQ-5D-5L, moderate to extreme problems							
Mobility	6 (15.6%)	5 (16.1%)	1 (7.1%)	0.39	2 (15.4%)	4 (12.5%)	0.36
Self-care	6 (15.6%)	6 (19.4%)	0	0.11	3 (23.1%)	3 (9.4%)	0.62
Usual activities	23 (51.1%)	19 (61.3%)	4 (28.6%)	0.09	8 (61.5%)	15 (46.9%)	0.11
Pain and discomfort	14 (31.1%)	12 (38.7%)	2 (14.3%)	0.12	4 (30.8%)	10 (31.3%)	0.95
Anxiety and depression	17 (37.8%)	13 (41.9%)	4 (28.6%)	0.71	5 (38.5%)	12 (37.5%)	0.43
WHODAS 2.0, moderate to extreme difficulty							
Cognition							
Learning a new task	5 (11.1%)	3 (9.7%)	2 (14.3%)	0.43	1 (7.7%)	4 (12.5%)	0.36
Concentrating on doing something	4 (8.9%)	3 (9.7%)	1 (7.1%)	0.70	1 (7.7%)	3 (9.4%)	0.66
Mobility							
Standing for long periods	19 (42.2%)	15 (48.4%)	4 (28.6%)	0.04	6 (46.2%)	13 (40.6%)	0.39
Walking a long distance	18 (40.0%)	15 (48.4%)	3 (21.4%)	0.02	6 (46.2%)	12 (37.5%)	0.20
Self-care							
Washing their whole bod	6 (13.3%)	6 (19.4%)	0	0.13	3 (23.1%)	3 (9.4%)	0.20
Getting dressed	5 (11.1%)	5 (16.1%)	0	0.09	3 (23.1%)	2 (6.3%)	0.28
Getting along							
Dealing with people	5 (11.1%)	2 (6.5%)	3 (21.4%)	0.21	1 (7.7%)	4 (12.5%)	0.92
Maintaining a friendship	3 (6.7%)	1 (3.2%)	2 (14.3%)	0.61	0	3 (9.4%)	0.60
Life activities							
Taking care for responsibilities	14 (31.1%)	11 (35.5%)	3 (21.4%)	0.20	4 (30.8%)	10 (31.3%)	0.47
Day-to-day work responsibilities	17 (37.8%)	14 (45.2%)	3 (21.4%)	0.02	6 (46.2%)	11 (34.4%)	0.08
Participation							
Community activities	12 (26.7%)	10 (32.3%)	2 (14.3%)	0.06	4 (30.8%)	8 (25.0%)	0.65
Emotionally affected by health problems	23 (51.1%)	15 (48.4%)	8 (57.1%)	0.31	5 (38.5%)	18 (56.3%)	0.12

EQ-5D-5L – EuroQol five-dimension five-level questionnaire. WHODAS 2.0 – World Health Organization Disability Assessment Schedule 2.0, 12-question questionnaire. IMV – invasive mechanical ventilation

Renmin Hospital of Wuhan University (Wuhan, China) showed that 23% had psychosocial symptoms and 18% sleep disorders.[18] Regarding critical COVID-19, Huang C *et al* reported that 41% had pain/discomfort problems and 32% anxiety/depression in the Eq-5D-5D questionnaire,[9] which was similar to our results.

The global self-perception of quality of life was positive with a median EQ-VAS score of 75.0 (60.0-90.0), which was also similar to the results of Huang C *et al*.[9] The EQ-5D-5L questionnaire reflects the perception of patientsthat was evaluated during a pandemic period where a significant part of the world's population was in isolation, having few social interactions and engaging mostly in controlled outdoor activities, which may have influenced the survivors' perception of disability. In contrast, the WHODAS 2.0 questions are more objective in evaluating the disability degree and that may explain why we found significant differences regarding mobility domain between IMV and no-IMV patients. On the other hand, almost one third of patients we studied did not need deep sedation and invasive ventilation which may influence favorable disability results. Dexmedetomidine was used in 60% of the patients for avoidance or early control of agitation and has been associated with a reduction in the duration of mechanical ventilation, delirium, intensive care length of stay and incidence of PICS.[19]

As recognized in the literature, survivors of critical illness often present a delayed return to work, with approximately two-thirds remaining on sick leave up to three months following hospital discharge, two-fifths up to 12 months and one-third up to 60 months.[20] Among the 19 previously employed survivors who participated in this study, 15 (78.9%) were still on sick leave and only four (21.1%) had returned to regular work.

We acknowledge several limitations to our study. First, this is a single center retrospective study with a small population of severe COVID-19. Second, the follow-up evaluation period was heterogeneous, having occurred between the 30th and 90th day after hospital discharge. Third, the applied scales reflect patients perception about the degree of their disability. Fourth, disability was evaluated during a pandemic period and the lockdown may have led to an over estimation of quality of life by the patients themselves. However, we believe this study reflects new data about the importance of clinical focus on functional outcomes in COVID-19 critically ill patients and the importance of an organized post-critical illness response to these survivors.

CONCLUSION

Health-related quality of life and disability assessment in COVID-19 survivors must be a priority. Activities associated with outdoor practices and interpersonal interaction were the most affected patient-reported outcomes with an important impact in anxiety disorders. The EQ-5D-5L questionnaire reported the highest incidence of moderate to extreme problems in usual activities, anxiety/depression and pain/discomfort. The disability assessment using the WHODAS 2.0 questionnaire showed that mobility, life activities and participation were the most affected domains. An organized follow-up structure in Intensive Care Medicine Departments and the recognition of the main impairments inherent to this kind of patients has the potential to improve functional and health-related outcomes in COVID-19 survivors and their families.

AUTHORS CONTRIBUTION

JF: Contribution to the design and draft of the work. Analysis and interpretation of data. Draft of the paper, critical review and final approval of the version to be published.

LF, IC, JAP: Contribution to the design and draft of the work. Analysis and interpretation of data. Critical review and final approval of the version to be published.

PROTECTION OF HUMAN SUBJECTS

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 issued by 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 no conflicts of interest to declare.

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REFERENCES

1. Direção Geral da Saúde, República Portuguesa. Casos de infeção por novo coronavírus (COVID-19). 2020. [accessed 2020 Oct 10]. Available from: https://covid19.min-saude.pt/wp-content/uploads/2020/03/Atualiza%C3%A7%C3%A3o-de-02032020-1728.pdf.
2. Stam HJ, Stucki G, Bickenbach J. Covid-19 and Post Intensive Care Syndrome: A Call for Action. J Rehabil Med. 2020;52:jrm00044.
3. Grasselli G, Pesenti A, Cecconi M. Critical Care Utilization for the COVID-19 Outbreak in Lombardy, Italy: early experience and forecast during an emergency response. JAMA. 2020;323:1545-6.
4. Rodriguez-Morales A, Cardona-Ospina J, Gutiérrez-Ocampo E, Villamizar-Peña R, Holguin-Rivera Y, Escalera-Antezana JP, et al. Clinical, laboratory and imaging features of COVID-19: A systematic review and meta-analysis. Travel Med Infect Dis. 2020;34:101623.
5. Needham DM, Davidson J, Cohen H, Hopkins RO, Weinert C, Wunsch H, et al. Improving long-term outcomes after discharge from intensive care unit: report from a stakeholders' conference. Crit Care Med. 2012;40:502-9.
6. Marra A, Pandharipande PP, Girard TD, Patel MB, Hughes CG, Jackson JC, Thompson JL, Chandrasekhar R, Ely EW, Brummel NE. Co-occurrence of post-intensive care syndrome problems among 406 survivors of critical illness. Crit Care Med. 2018;46:1393-401.
7. Hodgson CL, Udy AA, Bailey M, Barret J, Bellomo R, Bucknall T, et al. The impact of disability in survivors of critical illness. Intensive Care Med. 2017;43:992-1001.

8. Jaffri A, Jaffri UA. Post-Intensive care syndrome and COVID-19: crisis after a crisis? Heart Lung. 2020;49:883-4.
9. Huang C, Huang L, Wang Y, Li X, Ren L, Gu X, et al. 6-month consequences of COVID-19 in patients discharged from hospital: a cohort study. Lancet. 2021;397:220–32.
10. EuroQol Research Foundation. EQ-5D-5L User Guide, version 3.2019. [accessed 2020 Oct 28]. Available from: https://euroqol.org/publications/user-guides.
11. Ferreira PL, Antunes P, Ferreira LN, Pereira LN, Ramos-Goñi JM. A hybrid modelling approach for eliciting health state preferences: the Portuguese EQ-5D-5L value set. Qual Life Res. 2019;28:3163-75.
12. Ustün TB, Chatterji S, Kostanjsek N, Rehm J, Kennedy C, Epping-Jordan J, et al. WHO/NIH Joint Project. Developing the World Health Organization Disability Assessment Schedule 2.0. Bull World Health Organ. 2010;88:815-23.
13. Castro SS, Leite CF. Avaliação de Saúde e Deficiência – Manual do WHO Disability Assessment Schedule (WHODAS 2.0). Organização Mundial da Saúde. 2015 April. [accessed 2020 Oct 28]. Available from: https://apps.who.int/iris/bitstream/handle/10665/43974/9788562599514_por.pdf;jsessionid=9AA24E19FAA42010BF2233FB9E628D9C?sequence=19.
14. Fan E, Dowdy DW, Colantuoni E, Mnendez-Tellez PA, Severansky JE, Shanholtz C, et al. Physical complications in acute lung injury survivors: a 2-year longitudinal prospective study. Crit Care Med. 2014;42:849-59.
15. Grasselli G, Zangrillo A, Zanella A, Antonelli M, Cabrini L, Castelli A, et al. Baseline Characteristics and Outcomes of 1591 Patients Infected With SARS-CoV-2 Admitted to ICUs of the Lombardy Region, Italy. JAMA. 2020;323:1574–81.
16. Cody ND, Lakey SM, McMahon SM, Downey MK, Duncan MS, Hewitt JA, et al. Clinical characteristics and post-intensive care outcomes of COVID-19 pneumonia. 2020 [cited 2020 Oct 12]. Available from:. https://doi.org/10.21203/rs.3.rs-58685/v1.
17. Hosey MM, Needham DM. Survivorship after COVID-19 ICU stay. Nat Rev Dis Primers. 2020;6:60.
18. Xiong Q, Xu M, Li J, Liu Y, Zhang J, Xu Y, Dong W. Clinical sequelae of COVID-19 survivors in Wuhan, China: a single-centre longitudinal study. Clin Microbiol Infect. 2021;27:89-95.
19. Devlin JW, Skrobik Y, Gélinas C, Needham DM, Slooter AC, Pandharipande PP. Clinical practice guidelines for the prevention and management of pain, agitation/sedation, delirium, immobility, and sleep disruption in adult patients in the ICU. Crit Care Med. 2018;46:e825-73.
20. Kamdar BB, Suri R, Suchyta MR, Digrande KF, Sherwood KD, Colantuoni E, et al. Return to work after critical illness: a systematic review and meta-analysis. Thorax. 2020;75:17-27.

Cardiovascular Complications of COVID-19 Infection

Complicações Cardiovasculares Associadas à Infeção por COVID-19

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ABSTRACT

Introduction: Reports of cardiovascular complications related to the COVID-19 infection have been frequent.

Methods: Narrative review for relevant articles on the topic. The classic cardiovascular risk factors, like age, obesity, diabetes, and hypertension are associated with adverse outcomes in COVID-19 patients. Cardiovascular complications can have a diverse clinical presentation including silent myocardial injury, acute coronary syndromes, thromboembolism, cardiac arrhythmias, and heart failure. There are multiple mechanisms of cardiac injury that are not mutually exclusive. The approach to diagnosis and management should be carried out according to usual practice, while considering the particularities of COVID-19 infection.

Conclusion: The interaction between SARS-CoV-2 and the heart is complex and is manifested in multiple ways. Regardless of the clinical presentation, cardiac complications convey a worse prognosis. Patients should be actively monitored and treated accordingly.

Keywords: Arrhythmia; COVID-19; Heart Failure; Myocardial Infarction; Pulmonary Embolism; SARS-CoV-2

RESUMO

Introdução: As complicações cardiovasculares associadas a infeção por COVID-19 têm sido frequentemente reportadas.

Métodos: Revisão da literatura sobre os artigos relevantes neste tópico. Os fatores de risco cardiovasculares clássicos como idade, obesidade, diabetes e hipertensão foram associados a um risco maior de evolução desfavorável. As complicações cardiovasculares podem ter uma apresentação clínica variável incluindo lesão miocárdica, síndrome coronário agudo, trombo-embolismo, arritmias e insuficiência cardíaca. Estão descritos múltiplos mecanismos de lesão cardíaca os quais não são mutualmente exclusivos. A abordagem diagnóstica e terapêutica deve seguir a prática comum tendo, no entanto, em consideração as particularidades da infeção por COVID-19.

Conclusão: A interação entre a infeção por COVID-19 e o coração é complexa e manifesta-se de várias formas. Independentemente da apresentação clínica, as complicações cardíacas conferem um prognostico desfavorável pelo que devem ser monitorizadas ativamente e tratadas de forma apropriada.

Palavras-chave: Arritmia; COVID-19; Embolia Pulmonar; Enfarte do Miocárdio; Insuficiência Cardíaca

INTRODUCTION

As of the 1st of March of 2021, almost one year after the World Health Organization declared the COVID-19 viral infection as a pandemic, there were more than 113 million cases reported worldwide. The current global case fatality rate is 2.2%.[1,2] Cardiovascular disease and risk factors like obesity, diabetes and hypertension are associated with an increased risk of adverse outcomes from COVID-19 infection.[3] In a recent meta-analysis, the intensive care unit admission rate was 10.9%.[4] The typical presentation of severe COVID-19 infection is viral pneumonia. However, the clinical picture varies widely, from asymptomatic or mild flu-like symptoms to acute respiratory distress syndrome (ARDS) and multisystemic complications. Cardiovascular complications of COVID-19 infection are being reported more frequently and range from silent myocardial injury to acute coronary syndromes, thromboembolic events, arrhythmia, and cardiogenic shock.

The purpose of this clinical review is to merge current data on the most common cardiovascular complications associated to COVID-19 infection and their underlying mechanisms. Bearing in mind the evolving nature of this topic, and the present knowledge gaps, we aim to provide a clinical update of the most recent evidence.

METHODS

A narrative review using PubMed was conducted using the keywords 'COVID-19' or 'SARS-CoV-2' along with 'cardiovascular', 'cardiac', 'arrhythmia', 'myocardial injury', 'myocardial infarction', 'pulmonary embolism', 'out-of-hospital-cardiac-arrest' and 'heart failure'. Prospective and retrospective studies, reviews, meta-analysis, clinical guidelines, guidance documents and case reports, were included. Relevant articles published in English were screened and included only after critical review.

Risk factors for severe COVID-19 infection

Classic cardiovascular risk factors, namely, hypertension, diabetes and obesity, are frequent comorbidities in

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COVID-19 patients and convey an increased risk for adverse outcomes.[5-9] The prevalence of hypertension ranged from 15% - 17% in the Chinese population,[5,6] to 56% in the New York study.[9] Diabetes was present in 7% and 17%, respectively. Previous cardiovascular disease was reported in 14% to 35% of patients,[9,10] with one study specifically reporting that coronary artery disease was present in 2.5% of the overall population and 9% of the critically ill patients.[5]

The presence of these comorbidities confers an increased risk of developing severe forms of COVID-19 infection: the New York study highlighted obesity as a significant risk factor for mechanical ventilation.[9] A meta-analysis found that cardio or cerebrovascular disease increased the risk of developing severe manifestations of COVID-19 three-fold.[6] In a nationwide Danish study, risk factors for death included age [odds ratio (OR) 15 (95% CI 7.6 - 24) for 70 - 79 years, when compared with the 50 - 59 years age group], male sex, comorbidities and chronic diseases.[8] Likewise, the presence of ischemic heart disease and heart failure (HF) increased the risk of hospitalization (OR 1.4, 95% CI 1.2 – 1.7) and 2.2 95% CI 1.7 - 2.9, respectively).

Pathophysiological mechanisms

There is consensus that endothelial dysfunction is the final and most relevant pathological lesion caused by COVID-19 infection.[11-14] The SARS-CoV-2 virus enters host cells via angiotensin conversion enzyme 2 (ACE 2) receptors on the cellular membrane, which is abundant in the lungs, heart and vessels. The SARS-CoV-2 virus triggers a generalized inflammatory response, via dysregulation of multiple pathways that coordinate host defence systems and homeostasis. In severe cases, patients can develop a feedback loop that perpetuates inflammation and aggravates endothelial damage.[11]

Mechanisms of cardiac injury include cytokine-mediated immune activation, direct cardiotoxicity, micro and macrovascular dysfunction, and hypercoagulability. The risk of myocardial injury is significant and multifactorial, either by destabilization of coronary plaques, vasospasm, thromboembolic events, hypoxic lesion leading to type 2 myocardial infarction (MI), catecholamines-induced cardiomyopathy or myocarditis.[12-14]

From a hemodynamic standpoint, COVID-19 infection causes multiple pressure and volume changes that directly affect the heart. Severe pulmonary infection increases pulmonary pressures and therefore right ventricle (RV) afterload. Positive pressure ventilation also increases RV afterload and decreases venous return (RV preload). The RV responds by increasing contractility and dilating to increase its end-diastolic volume and thus its stroke volume (Frank-Starling law). However, it fares poorly with acute increases in pressure and these mechanisms can quickly fail. The systemic cardiac output can be compromised both by decreased left ventricle (LV) preload (due to decreased venous return, RV failure and paradoxical septal movement that reduces LV filling in diastole) and impaired LV contractility caused by hypoxia, inflammation, or other 'direct' cardiac injuries, like coronary occlusion, spasm or myocarditis.

Severe inflammation can also occur, causing vasoplegia, and manifesting with hypotension. On a physiological level, decreased systemic vascular resistance, combined with changes in capillary permeability, lead to fluid leaks to the extravascular compartment and cause reduction in venous return, as well as edema.

Low cardiac output combined with hypotension can cause impairment in end-organ perfusion, which is required to define shock. In the kidneys, low perfusion will activate the renin-angiotensin-aldosterone system (RAAS). The failing heart cannot cope with the extra fluid retention and increased end-diastolic pressures induced by the RAAS and enters a deleterious loop that potentiates shock development.

In patients with severe pulmonary infection and ARDS, endothelial dysfunction leads to inflammatory lung edema, reduced lung compliance and ventilation/perfusion mismatch. If there is associated HF, the increase in end-diastolic pressure will be transmitted to the lung causing transudative edema. The 'cardiogenic' edema in a patient with severe lung injury compromises even further gas exchange and lung compliance.

Cardiac complications of COVID-19 infection

Myocardial injury

Myocardial injury in infected patients can occur by the multiple mechanisms mentioned earlier. It is expressed quantitatively by the release of troponin. Troponin elevation occurs in 17% to 22% of hospitalized patients with COVID-19 infection.[15,16] Myocardial injury is not a clinical diagnosis in itself, but troponin elevation is related to adverse prognosis in early observational studies.[10,16] A subsequent study using Cox regression found an increased risk of death in patients with cardiac injury [HR 4.2 (95% CI, 1.92 - 9.49)].[15] A review suggests monitoring troponin at baseline for all patients, and then every 48 hours in intermediate-high risk patients. While this might help risk stratification and provide clues to a potential cardiac complication, the document also emphasizes the need for good clinical judgment when interpreting the results.[17]

Myocarditis

The diagnosis of myocarditis involves well defined criteria from four different aspects: symptoms, EKG, troponin elevation and imaging [echocardiography and cardiac magnetic resonance (CMR)].[18] Coronary disease should be discarded in all patients with suspected myocarditis. Endomyocardial biopsy is a class I indication in persisting severe HF and fulminant myocarditis.

In COVID-19 patients, myocarditis can be challenging to diagnose due to overlapping symptoms (dyspnoea and chest pain from either cardiac or pulmonary origin) and difficulties in obtaining CMR, which is of paramount importance to confirm the diagnosis.

Considering the pathophysiology of the COVID-19 infection, especially in critically ill patients, it is likely that

myocarditis is more prevalent than reported. The underlying specific mechanisms of COVID-19 myocarditis are not clearly defined, although they are thought to be mediated by activated T-cells and macrophages that infiltrate the myocardium.[12,13] The cytokine storm phase of COVID-19 infection further increases the risk of severe cardiac damage and arrhythmias. Furthermore, a high viral load has been associated with fulminant myocarditis in these patients.[19] A meta-summary of cases found 12 examples of CMR or biopsy proven COVID-19 myocarditis in the literature.[20] The most common symptoms were fever, dyspnoea and chest pain. EKG changes included T-wave changes and ventricular tachycardia. LV dysfunction was also frequent. Severe myocarditis was associated with cytokine storm. Patients were treated with drugs for HF, as well as a variety of immunosuppressants, glucocorticoids and IV immunoglobulins.[21,22] However, none of these treatments has been tested in specific trials.

The timing of presentation is currently uncertain, with reports of symptoms weeks after the COVID-19 infection.[23] Furthermore, histological evidence of COVID-19 inside the cardiomyocyte is unavailable, although one report found viral particles outside the cardiomyocytes.[24]

Acute coronary syndrome

Acute coronary syndrome (ACS) may be the initial presentation of COVID-19 or may complicate the course of an infected patient. Data is scarce about the incidence of ACS prompted by COVID-19 infection.

Importantly, the general ACS admission rate (non-COVID related) during the first wave of the pandemic, decreased up to 54% according to an international report.[25] Likewise, a reduction in coronary angiography and other diagnostic tests was noted.[26] Possible reasons for this include late admissions of patients due to fear of coming to the hospital and overwhelmed healthcare systems with decreased capacity to provide care. A 30 minute increase in door-to-guidewire time was also found in one study.[27] Difficulties in defining circuits for COVID-19 patients and managing the additional time needed for personnel and catheterization laboratory preparation contribute to the delays. The decrease in procedures was more substantial in low to middle income countries.[26] Adverse outcomes like in-hospital death, cardiogenic shock, ventricular arrhythmias and need for mechanical support were also more common during the first wave.[27]

Clinical presentation of suspected ACS in COVID-19 patients may range from ST-elevation MI to MI with non-obstructive coronary arteries (known as MINOCA, which is a working diagnosis for multiple possible causes from myocarditis to coronary dissection) and type 2 MI (which is typically caused by a mismatch between oxygen supply and demand, and not related to an acute coronary plaque event like ACS). As for angiographic findings, several studies found a higher prevalence of nonobstructive disease from 33% to 40% in those who underwent cardiac catheterization for suspected ST-elevation MI.[28,29]

In patients with unclear diagnosis, close clinical monitoring and serial EKG and troponin measurements should be performed. EKG changes over time, the pattern of rise in cardiac biomarkers as well as segmental wall changes in echocardiography are critical to define the best course of action.

Management of ACS should follow the appropriate guidelines for ST-elevation and non-ST elevation coronary syndromes. The European Society of Cardiology (ESC) has developed a guidance document with useful practical considerations in the specific context of the COVID-19 pandemic.[14] For ST-elevation MI, all efforts must be employed to achieve primary percutaneous coronary intervention (PCI) as soon as possible. Fibrinolysis is reserved for eligible patient who cannot timely reach a PCI centre. For non-ST elevation MI patients with intermediate risk (diabetes, renal insufficiency, early post-infarction angina or previous PCI or coronary bypass) the ESC guidance document suggests considering non-invasive testing, depending on availability and expertise.

Heart failure

Data on acute HF prevalence in COVID-19 patients is limited. Zhou *et al* found that 23% of hospitalized patients developed symptoms of HF and was associated with poorer prognosis.[16]

As for ACS, a striking decrease in the admission rate for HF during the first wave was noticed.[30,31] This raises concerns for patient safety and potential adverse outcomes. However, the specific consequences and impact in HF patients are not yet reported. Telemedicine programs have been suggested as an effective tool to help mitigate this issue.[32]

The underlying causes for acute HF in the COVID-19 patient are numerous and not mutually exclusive. These include ARDS, hypoxic lesions, hypervolemia, cardiorenal syndromes and pulmonary embolism, but also specific cardiac complications, such as MI, myocarditis, stress-induced cardiomyopathy and arrhythmias.[14,33,34] Furthermore, any of these mechanisms can result in an acute decompensation of a chronic HF patient.

Defining hemodynamic profiles is more important for the management of the COVID-19 critical patient than phenotypical HF classifications (reduced or preserved ejection fraction). Integrating clinical assessment with laboratory values, EKG and echocardiographic findings is key, both to identify reversible causes that require specific treatment (like ACS or arrhythmia) and to define the best therapeutic strategy.[34] Meticulous assessment of volume status and perfusion should be done using multiple parameters: clinical (peripheral perfusion, edema, pulmonary congestion, urinary output, central venous pressure), laboratory (cardiac biomarkers, central venous O_2 saturation, lactate, markers of liver and renal dysfunction) and imaging (mainly cardiac and pulmonary ultrasound). Hypervolemia is best managed with diuretics (while also avoiding hypovolemia, especially in vasoplegic states and in RV failure).

If cardiogenic shock ensues, starting inotropes with pulmonary vasodilatory properties may be useful. In low blood pressure states, especially with compromised renal perfusion and congested venous system, vasopressors are often necessary. HF drugs should be carefully considered in each case. Measurement of NT-proBNP is useful and has prognostic value.[35]

Of note, despite initial controversies with the use of ACE-inhibitors, several large cohort studies,[36,37] and a randomized trial[38] have found no association between ACE inhibitors and adverse outcomes.

Stress cardiomyopathy

Stress cardiomyopathy (stress CMP) or Takotsubo syndrome has rarely been reported as a complication of COVID-19 infection. In a case series,[39] 16 reports were found in the literature. Both genders were equally affected and mean age was 57 years. Most patients had reduced LV ejection fraction and three patients died. These characteristics differ from non-COVID-19 related stress CMP that typically affect postmenopausal women and rarely causes death. Interestingly, some studies have reported also an increase in stress CMP in non-infected patients. A Cleveland clinic study of patients with suspected ACS and a negative test for COVID-19, found a 4.58-fold increase in stress CMP compared to the pre-pandemic period.[40]

Stress CMP is characterized by reversible ventricular dysfunction, and the most typical pattern is 'apical ballooning' with akinesia of all distal segments of the LV walls. It is usually caused by an emotional or physical trigger. Evaluation of coronary disease is mandatory in all patients, by coronary angiography or computed tomography (CT).[41] Accepted pathological mechanisms for stress CMP include high circulating catecholamine levels, exaggerated inflammatory response and direct cytotoxicity.[39,41]

Treatment depends on the severity of presentation and is mainly supportive. In cardiogenic shock, non-aminergic inotropic drugs may be a good option. However, in some cases, patients can develop an obstruction of the LV outflow tract due to hypercontractility of the basal portions. In this scenario, the use of inotropes may be counterproductive.[42]

Pericarditis and pericardial effusion

Isolated pericarditis has been infrequently reported in relation with COVID-19 infection. However, pericardial effusion accompanying myocarditis has been reported in up to 50% of cases.[20] In the available case reports for isolated pericarditis, the first clue to the diagnosis was the typical chest pain.[43,44] Evolution was usually benign. Regarding treatment, experts recommend maintaining colchicine, while reserving ibuprofen for worsening pain. For fever and other systemic symptoms, paracetamol is preferred. Furthermore, corticosteroids and anakinra (a recombinant modified version of the interleukin one receptor antagonist protein) can be considered to treat both situations.[45]

Cardiac arrhythmias

Cardiac arrhythmias have been described in 17% of the hospitalized cases and in almost 50% of patients in the intensive care units.[46] Cardiac arrhythmias probably contribute to a higher risk of adverse outcomes, with an increased risk of in-hospital death, and varies from bradyarrhythmia (less common), to sinus tachycardia and atrial arrhythmias (the most frequently reported), like atrial fibrillation (AF) and atrial flutter, and ventricular dysrhythmias, including cardiac arrest.[47] In infected hospitalized patients, continuous EKG monitoring and accurate and prompt recognition of arrhythmias are important.

AF is one of the common chronic comorbidities found in patient deaths due to COVID-19.[48] In a large propensity score matched study of COVID-19 patients aged over 50 years of age, AF significantly increased short-term mortality and thromboembolic events.[49]

Risk factors for the development of cardiac arrhythmias are: 1. Respiratory failure, 2. myocardial ischemia or myocarditis, 3. cardiogenic shock, 4. sepsis or systemic inflammation, 5. hyperactivity of sympathetic nervous system, 6. hypercoagulability status, 7. electrolyte disturbances, 8. Therefore, the incidence of cardiac arrest and cardiac arrhythmias in patients with COVID-19 are likely the consequence of systemic illness and not solely the direct effect of the viral infection. In a study assessing the QT interval in COVID-19 patients, the hydroxychloroquine-azithromycin combination regimen was not associated with arrhythmic fatalities and none of the deceased had a QTc over 500 ms.[50] In an early report from Guo *et al*, among 187 patients with COVID-19 from a Chinese cohort, 5.9% had ventricular tachyarrhythmias while hospitalized.[10]

Out-of-hospital cardiac arrest

Since the onset of the COVID-19 pandemic an increase in the incidence of out-of-hospital-cardiac-arrest (OHCA) has been consistently reported worldwide.[51-54] In an Italian study, COVID-19 diagnosis accounted for around 80% of these events.[51] This rise in OHCA is presumably due to a combination of acute respiratory failure and pulmonary embolism associated with COVID-19, and arrhythmic sudden death due to myocardial injury and QT interval prolongation (infection-related or caused by administration of drugs to treat the infection). OHCA has been accompanied by a reduction in survival from resuscitation efforts. Potential causes include delay in seeking medical care, longer waiting times for emergency services, fewer shockable rhythms and lower rates of bystander initiated cardiopulmonary resuscitation.

Pulmonary embolism

Pulmonary embolism (PE) is one of the most severe thromboembolic manifestations. Recent studies report overall incidences of PE in COVID-19 patients between 1.1% - 3.4%,[55,56] but rising up to 17% - 27% in critical patients.[57,58] In an autopsy study, PE was the direct cause of death in 33% of COVID-19 patients.[59] These rates are

higher than previous reports for other infections. For instance for H1N1 influenza, the rate of venous thromboembolism was approximately 6%.[60] The mechanism of thromboembolic events in COVID-19 infection is not fully understood, but all three components of the Virchow triad (endothelial injury, stasis, and hypercoagulable state) are thought to play a major role in thrombus formation. Coagulation abnormalities are frequent in COVID-19 patients,[61,62] and are associated with adverse outcomes.[62,63] Remarkably, deep venous thrombosis (DVT) has seldom been reported as the cause for PE. Although this might be partially explained by suboptimal screening, some studies suggest that pulmonary *in situ* thrombosis instead of embolism is also present.[64]

Several expert consensus agree on the following recommendations[62,65-68]: 1. Thromboprophylaxis (mechanical or pharmacological) in hospitalized patients; 2. Serial monitoring of haemostasis through platelet count, prothrombin time, fibrinogen and d-dimers in intensive care unit patients; 3. In patients under therapeutic anticoagulation, monitoring of anti-Xa levels is recommended every 48 hours; 4. Unfractionated heparin or low molecular weight heparin is preferred to fondaparinux or direct oral anticoagulants; 5. Extending prophylactic anticoagulation after hospital discharge in patients with low bleeding risk should be considered.

French experts suggest using an intermediate anticoagulation dose for patients with a body mass index (BMI) over 30 kg/m^2 or with risk factors for DVT. For those with D-dimer levels over 3 ug/mL and fibrinogen over 8 g/L the recommendation is to use the full anticoagulation dose.[65] A Mayo clinic review proposes an intermediate anticoagulation dose for those with BMI over 40 kg/m^2 or D-dimer over 3 ug/mL.[62] These recommendations are controversial due to lack of controlled studies and lack of mention in other expert consensual documents.[66,67]Observational studies in critical COVID-19 patients found associations between higher dose anticoagulation and a lower risk of death, with a non-significant increase in major bleeding.[69,70] However, this was not observed in a systematic review and meta-analysis.[62]

In COVID-19 patients, an abrupt deterioration in oxygenation should raise suspicion for PE, especially if accompanied by sudden hypotension and tachycardia. If CT scan is not feasible, bedside echocardiogram should be performed to assess for cardiac signs of PE.[71] Yet, the interpretation of echocardiogram findings is ARDS patients can be challenging, especially if there are no previous tests to compare with. Multidisciplinary discussion, integrating all clinical information, is the best strategy for decision making. In patients where PE seems the most likely cause of obstructive shock, thrombolysis should be considered.[61,71] In patients with suspected PE who are not in shock or bedridden, angio-CT scan is the gold standard. These patients have an indication for full-dose anticoagulation and should be closely monitored for signs of decompensation.[71]

CONCLUSION

Cardiac complications of COVID-19 are frequent and can weigh heavily on prognosis. Diagnosis is challenging due to confounding factors and logistical hurdles. Multiparametric evaluation of patients, close monitoring and swift action when needed, are of paramount importance. We are only beginning to understand the wider implications of COVID-19 complications. The direct consequences of COVID-19 are becoming clearer, but we should also be wary of the indirect impact, caused by saturation of healthcare systems and decreased response capacity for many months.

AUTHORS CONTRIBUTION

MT: Conception of the work. Draft of the paper. Critical review of the manuscript.

AHR: Conception of the work. Critical review of the manuscript.

MO: Conception of the work. Draft of the paper. Critical review of the manuscript. Supervision and final approval.

COMPETING INTERESTS

The authors have declared that no competing interests exist.

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REFERENCES

1. World Health Organization. WHO Coronavirus Disease (COVID-19) Dashboard. 2020. [accessed 2021 Mar 01]. Available from: https://covid19.who.int/?gclid=EAIaIQobChMI65aDxPW57QIVEJntCh3SxgWwEAAYASAAEgLOZvD_BwE.
2. Zhou P, Yang XL, Wang XG, Hu B, Zhang L, Zhang W, et al. L. A pneumonia outbreak associated with a new coronavirus of probable bat origin. Nature. 2020;579:270-3.
3. Madjid M, Safavi-Naeini P, Solomon SD, Vardeny O. Potential effects of coronaviruses on the cardiovascular system: a review. JAMA Cardiol. 2020;5:831-40.
4. Zhang JJ, Lee KS, Ang LW, Leo YS, Young BE. Risk factors for severe disease and efficacy of treatment in patients infected with COVID-19: a systematic review, meta-analysis, and meta-regression analysis. Clin Infect Dis. 2020;71:2199-206.
5. Guan WJ, Ni ZY, Hu Y, Liang WH, Ou CQ, He JX, et al. Clinical characteristics of coronavirus disease 2019 in China. N Engl J Med. 2020;382:1708-20.
6. Li B, Yang J, Zhao F, Zhi L, Wang X, Liu L, et al. Prevalence and impact of cardiovascular metabolic diseases on COVID-19 in China. Clin Res Cardiol. 2020;109:531-8.
7. Grasselli G, Zangrillo A, Zanella A, Antonelli M, Cabrini L, Castelli A, et al. Baseline characteristics and outcomes of 1591 patients infected with SARS-CoV-2 admitted to ICUs of the Lombardy region, Italy. JAMA. 2020;323:1574-81.
8. Reilev M, Kristensen KB, Pottegard A, Lund LC, Hallas J, Ernst MT, et al. Characteristics and predictors of hospitalization and death in the first 11 122 cases with a positive RT-PCR test for SARS-CoV-2 in Denmark: a nationwide cohort. Int J Epidemiol. 2020;49:1468-81.
9. Richardson S, Hirsch J, Narasimhan M, Crawford J, McGinn T, Davidson KW, et al. Presenting characteristics, comorbidities, and outcomes

among 5700 patients hospitalized with COVID-19 in the New York city area. JAMA. 2020;323:2052-9.
10. Guo T, Fan Y, Chen M, Wu X, Zhang L, He T, et al. Cardiovascular implications of fatal outcomes of patients with coronavirus disease 2019 (COVID-19). JAMA Cardiol. 2020;5:811-8.
11. Libby P, Luscher T. COVID-19 is, in the end, an endothelial disease. Eur Heart J. 2020;41:3038-44.
12. Nishiga M, Wang DW, Han Y, Lewis DB, Wu JC. COVID-19 and cardiovascular disease: from basic mechanisms to clinical perspectives. Nat Rev Cardiol. 2020;17:543-58.
13. Guzik TJ, Mohiddin SA, Dimarco A, Patel V, Savvatis K, Marelli-Berg FM, et al. COVID-19 and the cardiovascular system: implications for risk assessment, diagnosis, and treatment options. Cardiovasc Res. 2020;116:1666-87.
14. European Society of Cardiology. ESC Guidance for the Diagnosis and Management of CV Disease during the COVID-19 Pandemic. 2020. [accessed 2020 Dec 15]. Available from: https://www.escardio.org/Education/COVID-19-and-Cardiology/ESC-COVID-19-Guidance.
15. Shi S, Qin M, Shen B, Cai Y, Liu T, Yang F, et al. Association of cardiac injury with mortality in hospitalized patients with COVID-19 in Wuhan, China. JAMA Cardiol. 2020;5:802-10.
16. Zhou F, Yu T, Du R, Fan G, Liu Y, Liu Z, et al. Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: a retrospective cohort study. Lancet. 2020;395:1054-62.
17. Sandoval Y, Januzzi JL Jr, Jaffe AS. Cardiac troponin for assessment of myocardial injury in COVID-19: JACC Review Topic of the Week. J Am Coll Cardiol. 2020;76:1244-58.
18. Caforio AL, Pankuweit S, Arbustini E, Basso C, Gimeno-Blanes J, Felix SB, et al. European Society of Cardiology Working Group on M and Pericardial D. Current state of knowledge on aetiology, diagnosis, management, and therapy of myocarditis: a position statement of the European Society of Cardiology Working Group on Myocardial and Pericardial Diseases. Eur Heart J. 2013;34:2636-48d.
19. Liu Y, Yang Y, Zhang C, Huang F, Wang F, Yuan J, et al. Clinical and biochemical indexes from 2019-nCoV infected patients linked to viral loads and lung injury. Sci China Life Sci. 2020;63:364-74.
20. Ho JS, Sia CH, Chan MY, Lin W, Wong RC. Coronavirus-induced myocarditis: a meta-summary of cases. Heart Lung. 2020;49:681-5.
21. Inciardi RM, Lupi L, Zaccone G, Italia L, Raffo M, Tomasoni D, et al. Cardiac involvement in a patient with coronavirus disease 2019 (COVID-19). JAMA Cardiol. 2020;5:819-24.
22. Hu H, Ma F, Wei X, Fang Y. Coronavirus fulminant myocarditis saved with glucocorticoid and human immunoglobulin. Eur Heart J. 2020;42:206.
23. Sardari A, Tabarsi P, Borhany H, Mohiaddin R, Houshmand G. Myocarditis detected after COVID-19 recovery. Eur Heart J Cardiovasc Imaging. 2020;22:131-2.
24. Tavazzi G, Pellegrini C, Maurelli M, Belliato M, Sciutti F, Bottazzi A, et al. Myocardial localization of coronavirus in COVID-19 cardiogenic shock. Eur J Heart Fail. 2020;22:911-5.
25. Araiza-Garaygordobil D, Montalto C, Martinez-Amezcua P, Cabello-Lopez A, Gopar-Nieto R, Alabrese R, et al. Impact of the COVID-19 pandemic on hospitalizations for acute coronary syndromes: a multinational study. QJM. 2021. Advance article. [accessed 2021 Fev 26]. Available from: https://pubmed.ncbi.nlm.nih.gov/33486512/.
26. Einstein AJ, Shaw LJ, Hirschfeld C, Williams MC, Villines TC, Better N, et al. International impact of COVID-19 on the diagnosis of heart disease. J Am Coll Cardiol. 2021;77:173-85.
27. Tam CF, Cheung KS, Lam S, Wong A, Yung A, Sze M, et al. Impact of coronavirus disease 2019 (COVID-19) outbreak on outcome of myocardial infarction in Hong Kong, China. Catheter Cardiovasc Interv. 2020;97:E194-7.
28. Bangalore S, Sharma A, Slotwiner A, Yatskar L, Harari R, Shah B, et al. ST-segment elevation in patients with Covid-19 - a case series. N Engl J Med. 2020;382:2478-80.
29. Stefanini GG, Montorfano M, Trabattoni D, Andreini D, Ferrante G, Ancona M, et al. ST-elevation myocardial infarction in patients with COVID-19: clinical and angiographic outcomes. Circulation. 2020;141:2113-6.
30. Barghash MH, Pinney SP. Heart failure in the COVID-19 pandemic: where has all New York's congestion gone? J Card Fail. 2020;26:477-8.
31. Andersson C, Gerds T, Fosbol E, Phelps M, Andersen J, Lamberts M, et al. Incidence of new-onset and worsening heart failure before and after the COVID-19 epidemic lockdown in Denmark: a nationwide cohort study. Circ Heart Fail. 2020;13:e007274.
32. Cleland JG, Clark RA, Pellicori P, Inglis SC. Caring for people with heart failure and many other medical problems through and beyond the COVID-19 pandemic: the advantages of universal access to home telemonitoring. Eur J Heart Fail. 2020;22:995-8.
33. Mehra MR, Ruschitzka F. COVID-19 illness and heart failure: a missing link? JACC Heart Fail. 2020;8:512-4.
34. Bader F, Manla Y, Atallah B, Starling RC. Heart failure and COVID-19. Heart Fail Rev. 2020;26:1-10.
35. Gao L, Jiang D, Wen XS, Cheng XC, Sun M, He B, et al. Prognostic value of NT-proBNP in patients with severe COVID-19. Respir Res. 2020;21:83.
36. Morales DR, Conover MM, You SC, Pratt N, Kostka K, Duarte-Salles T, et al. Renin-angiotensin system blockers and susceptibility to COVID-19: an international, open science, cohort analysis. Lancet Digit Health. 2021;3:e98-114.
37. Hippisley-Cox J, Young D, Coupland C, Channon KM, Tan PS, Harrison DA, et al. Risk of severe COVID-19 disease with ACE inhibitors and angiotensin receptor blockers: cohort study including 8.3 million people. Heart. 2020;106:1503-11.
38. Lopes R, Macedo A, de Barros E, Silva P, Moll-Bernardes R, Dos Santos TM, et al. Effect of discontinuing vs continuing angiotensin-converting enzyme inhibitors and angiotensin II receptor blockers on days alive and out of the hospital in patients admitted with COVID-19: a randomized clinical trial. JAMA. 2021;325:254-64.
39. Desai HD, Jadeja DM, Sharma K. Takotsubo syndrome a rare entity in patients with COVID-19: an updated review of case-reports and case-series. Int J Cardiol Heart Vasc. 2020;29:100604.
40. Jabri A, Kalra A, Kumar A, Alameh A, Adroja S, Bashir H, et al. Incidence of stress cardiomyopathy during the coronavirus disease 2019 pandemic. JAMA Netw Open. 2020;3:e2014780.
41. Ghadri J, Wittstein IS, Prasad A, Sharkey S, Dote K, Akashi YJ, et al. International Expert Consensus Document on Takotsubo Syndrome (Part I): Clinical Characteristics, Diagnostic Criteria, and Pathophysiology. Eur Heart J. 2018;39:2032-46.
42. Ghadri JR, Wittstein IS, Prasad A, Sharkey S, Dote K, Akashi YJ, et al. International Expert Consensus Document on Takotsubo Syndrome (Part II): Diagnostic Workup, Outcome, and Management. Eur Heart J. 2018;39:2047-62.
43. Faraj R, Belkhayat C, Bouchlarhem A, El Aidouni G, Bkiyar H, Housni B. Acute pericarditis revealing COVID-19 infection: case report. Ann Med Surg. 2021;62:225-7.
44. Kumar R, Kumar J, Daly C, Edroos S. Acute pericarditis as a primary presentation of COVID-19. BMJ Case Rep. 2020;13:e237617.
45. Imazio M, Brucato A, Lazaros G, Andreis A, Scarsi M, Klein A, et al. Anti-inflammatory therapies for pericardial diseases in the COVID-19 pandemic: safety and potentiality. J Cardiovasc Med. 2020;21:625-9.
46. Bhatla A, Mayer M, Adusumalli S, Hyman M, Oh E, Tierney A, et al. COVID-19 and cardiac arrhythmias. Heart Rhythm. 2020;17:1439-44.
47. Wang Y, Wang Z, Tse G, Zhang L, Wan EY, Guo Y, et al. Cardiac arrhythmias in patients with COVID-19. J Arrhythm. 2020;36:827-36.
48. Mai F, Del Pinto R, Ferri C. COVID-19 and cardiovascular diseases. J Cardiol. 2020;76:453-8.
49. Harrison S, Fazio-Eynullayeva S, Lane D, Underhill P, Lip G. Atrial fibrillation and the risk of 30-day incident thromboembolic events, and mortality in adults ≥ 50 years with COVID-19. J Arryhtmia. 2021;37:6.
50. Bernardini A, Ciconte G, Negro G, Rondine R, Mecarocci V, Viva T, et al. Assessing QT interval in COVID-19 patients: safety of hydroxychloroquine-azithromycin combination regimen. Int J Cardiol. 2020;324:242–8.
51. Baldi E, Sechi GM, Mare C, Canevari F, Brancaglione A, Primi R, et al, Lombardia CR. Out-of-hospital cardiac arrest during the Covid-19 outbreak in Italy. N Engl J Med. 2020;383:496-8.
52. Baldi E, Sechi G, Mare C, Canevari F, Brancaglione A, Primi R, et al. COVID-19 kills at home: the close relationship between the epidemic and the increase of out-of-hospital cardiac arrests. Eur Heart J. 2020;41:3045-54.
53. Fothergill RT, Smith AL, Wrigley F, Perkins GD. Out-of-hospital cardiac arrest in London during the COVID-19 pandemic. Resusc Plus. 2021;5:100066.
54. Lai PH, Lancet EA, Weiden MD, Webber MP, Zeig-Owens R, Hall CB et al. Characteristics associated with out-of-hospital cardiac arrests and resuscitations during the novel coronavirus disease 2019 pandemic in New York City. JAMA Cardiol. 2020;5:1154-63.
55. Grillet F, Behr J, Calame P, Aubry S, Delabrousse E. Acute pulmonary embolism associated with COVID-19 pneumonia detected with pulmonary CT angiography. Radiology. 2020;296:E186-8.
56. Leonard-Lorant I, Delabranche X, Severac F, Helms J, Pauzet C, Collange O, et al. Acute pulmonary embolism in patients with COVID-19

at CT angiography and relationship to d-dimer levels. Radiology. 2020;296:E189-91.
57. Beun R, Kusadasi N, Sikma M, Westerink J, Huisman A. Thromboembolic events and apparent heparin resistance in patients infected with SARS-CoV-2. Int J Lab Hematol. 2020;42:19-20.
58. Helms J, Tacquard C, Severac F, Leonard-Lorant I, Ohana M, Delabranche X, et al. High risk of thrombosis in patients with severe SARS-CoV-2 infection: a multicenter prospective cohort study. Intensive Care Med. 2020;46:1089-98.
59. Wichmann D, Sperhake JP, Lutgehetmann M, Steurer S, Edler C, Heinemann A, et al. Autopsy findings and venous thromboembolism in patients with COVID-19: a prospective cohort study. Ann Intern Med. 2020;173:268-77.
60. Bunce PE, High SM, Nadjafi M, Stanley K, Liles WC, Christian MD. Pandemic H1N1 influenza infection and vascular thrombosis. Clin Infect Dis. 2011;52:e14-7.
61. Bikdeli B, Madhavan MV, Jimenez D, Chuich T, Dreyfus I, Driggin E, et al. COVID-19 and thrombotic or thromboembolic disease: implications for prevention, antithrombotic therapy, and follow-up: JACC state-of-the-art review. J Am Coll Cardiol. 2020;75:2950-73.
62. McBane RD 2nd, Torres Roldan VD, Niven AS, Pruthi RK, Franco PM, Linderbaum JA, et al. Anticoagulation in COVID-19: a systematic review, meta-analysis, and rapid guidance from Mayo Clinic. Mayo Clin Proc. 2020;95:2467-86.
63. Tang N, Li D, Wang X, Sun Z. Abnormal coagulation parameters are associated with poor prognosis in patients with novel coronavirus pneumonia. J Thromb Haemost. 2020;18:844-7.
64. Sakr Y, Giovini M, Leone M, Pizzilli G, Kortgen A, Bauer M, et al. Pulmonary embolism in patients with coronavirus disease-2019 (COVID-19) pneumonia: a narrative review. Ann Intensive Care. 2020;10:124.
65. Susen S, Tacquard CA, Godon A, Mansour A, Garrigue D, Nguyen P, et al . Prevention of thrombotic risk in hospitalized patients with COVID-19 and hemostasis monitoring. Crit Care. 2020;24:364.
66. Marietta M, Ageno W, Artoni A, De Candia E, Gresele P, Marchetti M, et al. COVID-19 and haemostasis: a position paper from Italian Society on Thrombosis and Haemostasis (SISET). Blood Transfus. 2020;18:167-9.
67. Moores LK, Tritschler T, Brosnahan S, Carrier M, Collen JF, Doerschug K, et al. Prevention, diagnosis, and treatment of VTE in patients with coronavirus disease 2019: CHEST Guideline and Expert Panel Report. Chest. 2020;158:1143-63.
68. Spyropoulos AC, Levy JH, Ageno W, Connors JM, Hunt BJ, Iba T, et al. Scientific and Standardization Committee communication: clinical guidance on the diagnosis, prevention, and treatment of venous thromboembolism in hospitalized patients with COVID-19. J Thromb Haemost. 2020;18:1859-65.
69. Paranjpe I, Fuster V, Lala A, Russak AJ, Glicksberg BS, Levin MA, et al. Association of treatment dose anticoagulation with in-hospital survival among hospitalized patients with COVID-19. J Am Coll Cardiol. 2020;76:122-4.
70. Jonmarker S, Hollenberg J, Dahlberg M, Stackelberg O, Litorell J, Everhov AH, et al. Dosing of thromboprophylaxis and mortality in critically ill COVID-19 patients. Crit Care. 2020;24:653.
71. Konstantinides SV, Meyer G, Becattini C, Bueno H, Geersing GJ, Harjola VP, et al. 2019 ESC Guidelines for the Diagnosis and Management of Acute Pulmonary Embolism Developed in Collaboration with the European Respiratory Society (ERS). Eur Heart J. 2020;41:543-603.

Necrotizing Fasciitis Caused by Photobacterium damselae: The First Case in Portugal

Fasceíte Necrotizante Causada por Photobacterium damselae: O Primeiro Caso Descrito em Portugal

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ABSTRACT

Necrotizing fasciitis is a severe soft tissue infection with a high mortality rate and therefore requires emergent surgical treatment. Several microorganisms can cause this infection, *Photobacterium damselae* being one of them, with only eight cases previously published in the literature. We report the first ever case of necrotizing fasciitis, caused by this microorganism, in Portugal. In this case report the patient survived after several debridement procedures and reconstruction of the upper limb with acellular dermal matrix and skin graft. A brief review of the *Photobacterium damselae* soft tissue infection reports as well as the clinical presentation, diagnosis, pathophysiology and treatment of necrotizing fasciitis can also be found in this paper.

Keywords: Bacterial Infections; Fasciitis, Necrotizing; Hand Injuries; Photobacterium; Vibrio Infections

RESUMO

A fasceíte necrotizante é uma infeção grave que requer tratamento cirúrgico emergente, sendo responsável por uma elevada taxa de mortalidade. Existem vários microorganismos que podem ser responsáveis por este tipo de infecção, sendo o *Photobacterium damselae* um destes, com apenas oito casos descritos na literatura de fasceíte necrotizante por este agente, sendo o presente relato a primeira vez que é reportada uma infeção a este agente em Portugal. No presente caso o paciente sobreviveu após várias intervenções de desbridamento cirúrgico e reconstrução do membro superior com matriz dérmica acelular e enxertos de pele. Foi ainda realizada uma breve revisão de todos os relatos de infecção de tecidos moles por este agente, bem como um resumo da apresentação clínica, diagnóstico, fisiopatologia e tratamento da fasceíte necrotizante.

Palavras-chave: Fasceíte Necrotizante; Infecção por Vibrio; Infecções Bacterianas; Lesões da Mão; Photobacterium

INTRODUCTION

Necrotizing fasciitis is an infection involving the fascia and subcutaneous tissue, that spares the underlying tissues. Risk factors include immunosuppression, peripheral vascular disease, diabetes mellitus, chronic liver disease, and intravascular drug abuse.

Necrotizing fasciitis is often polymicrobial and group A β-hemolytic *Streptococcus* is the most common group of microorganisms identified in microbial culture tests.[1]

The subspecies *piscicida* of the bacterium *Photobacterium damselae* (*P. damselae*) is a well-studied fish pathogen, causing a zoonosis known as 'fish pasteurellosis',[2] but infections in human are rare. We describe the first ever infection by *P. damselae* reported in Portugal and make a brief literature review of both necrotizing fasciitis and *P. damselae infections.*

CASE DESCRIPTION

A 65 year old man, fisherman, was transferred to the emergency department of our hospital because of pain and edema in his right hand. There was history of chronic renal failure dependent on blood dialysis through an arterial-venous fistula in the right arm. His regular medication was lisinopril, alprazolam and bicalutamide.

On admission the patient complained of progressively worse right hand edema and severe pain. He mentioned a history of trauma 12 hours earlier in a fish cleaning table. During our physical examination he presented with right hand edema, reduced movement amplitude and strength of the wrist and all the fingers. The fingers had good perfusion and no sensitive deficits. There was a small wound in the dorsum of the hand (Fig. 1). The blood tests at admission had a white blood cell (WBC) count of 10 770 x 10^9/L with 86% neutrophils; and a C reactive protein (CRP) of 31.1 mg/L. Swab, skin biopsy and blood cultures were collected. About two hours after admission, the patient was taken to the operating room and fasciotomies of the hand and wrist were performed. Empiric antibacterial therapy with amoxicillin/clavulanic acid and metronidazole was initiated.

About 15 hours after admission, skin necrosis in the dorsum of the right hand and progression of edema to the forearm was observed. The patient developed septic shock and underwent emergent debridement (Fig. 2). A preliminary identification of *P. damselae* was possible after 48 hours and adequate antibiotic therapy with ceftriaxone and doxicicline was initiated.

On day 10 and 17 after admission, surgical debridement procedures were carried out. From the 17th day onwards, a regimen of negative pressure therapy was started. The antibiotic therapy was stopped at day 21. At day 25 an acellular dermal matrix (ADM) was applied (Fig. 3). Four weeks later,

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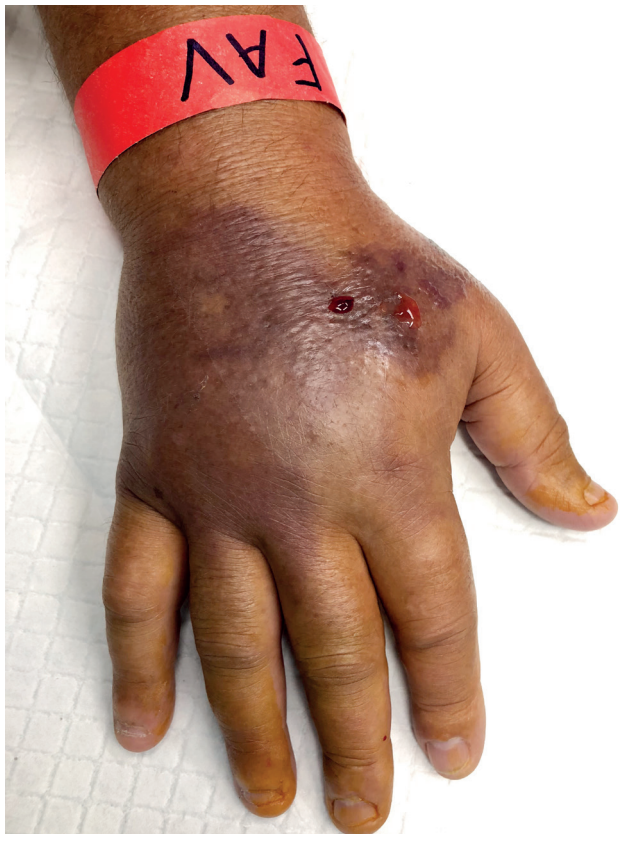

Figure 1 – Clinical presentation at admission, 12 hours after injury

the hand and forearm were skin grafted (Fig. 4). The patient was discharged from the hospital 70 days after admission after a total of six surgical interventions. A splint holding the wrist in extension was applied after ADM application until two weeks after skin graft. Daily physiotherapy was maintained for the next three months, and three times per week during the following six months. The patient was able to resume his daily life activities without restrictions. At present, he has a decreased amplitude of wrist and finger flexion (Fig. 5), but no extension restrictions.

DISCUSSION

Necrotizing fasciitis frequently appears only as low-grade cellulitis and can be very challenging to make an early diagnosis. The most common examination findings at the time of presentation are warmth (97%), erythema (95% – 100%), edema (82%) and disproportionate pain (98% – 100%).[1,3] Patients are frequently hemodynamically unstable with elevated WBC counts, coagulopathy, and shock.[4] Skin necrosis, *bullae*, crepitus, gas on imaging studies and hemodynamic instability, all suggestive signs of necrotizing fasciitis, are not always present. In fact one or more of these signs are present less than 50% of the time.[5] A more specific sign is a gray fat and liquified pus with 'dishwater' appearance along the fascial planes during debridement, while frank pus is uncommon.[6]

Laboratory markers can aid in diagnosis, sodium levels below 135 mmol/L and WBC greater than 15 400 cells/μL are the best predictors of necrotizing soft tissue infections.[5] The Laboratory Risk Indicator for Necrotizing Fasciitis (LRINEC) scale was developed in an attempt to help in the diagnosis.[7,8] A formal diagnosis of necrotizing fasciitis can be made on microscopic examination of biopsied fascia.[9]

An intensive care unit is recommended. Planned, staged debridements every 24 to 48 hours of affected limbs are expected, with an average of 3 – 4 debridements per patient.[1,10,11] Antibiotic therapy should be initiated empirically until retrieval of culture test results. The empirical antibiotic treatment should be broad (e.g, vancomycin or linezolid plus piperacillin-tazobactam or a carbapenem; or plus ceftriaxone and metronidazole), as the etiology can be polymicrobial.[12] Mortality from necrotizing fasciitis usually ranges from 23% to 76%, with organ failure and sepsis consisting in the major causes of death.[13] The factors mostly associated with increased mortality are delay in diagnosis, delay in surgical debridement, advanced age and having two or more comorbilities.[1]

In 1981 an 'unnamed marine *Vibrio*' was isolated as the

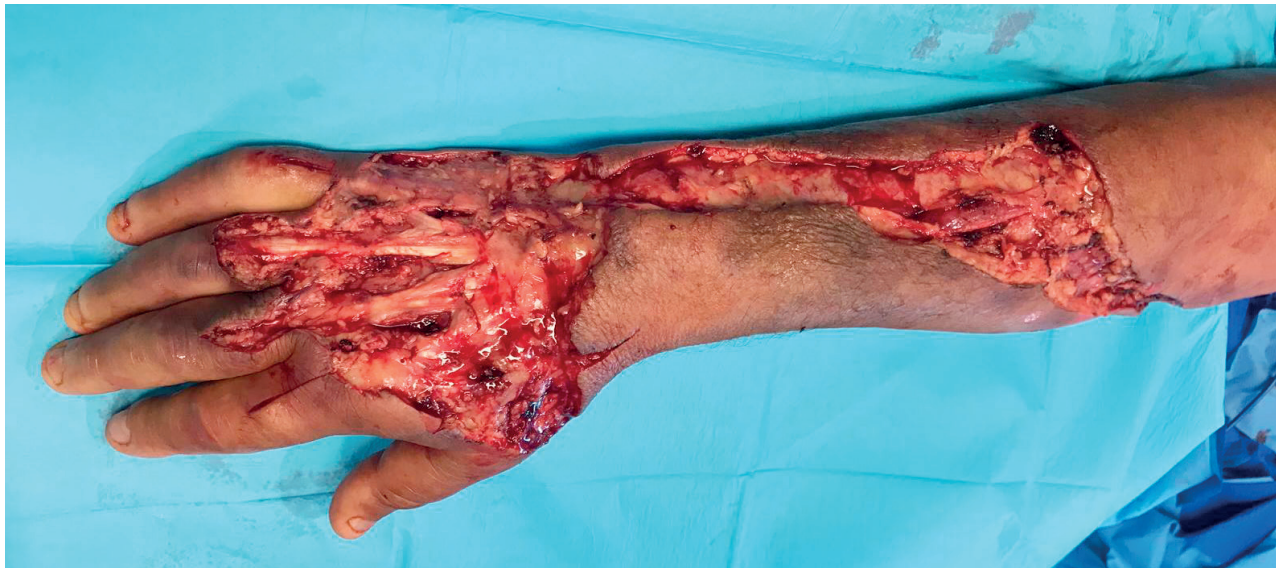

Figure 2 – Presentation after debridement

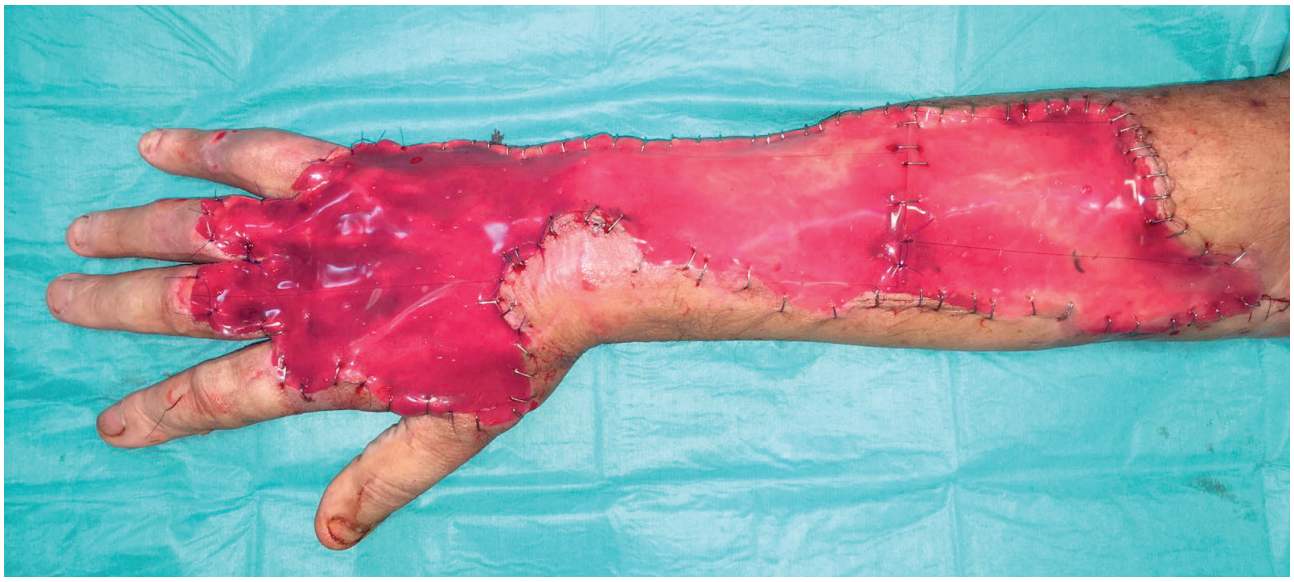

Figure 3 – Presentation after reconstruction with acellular dermal matrix

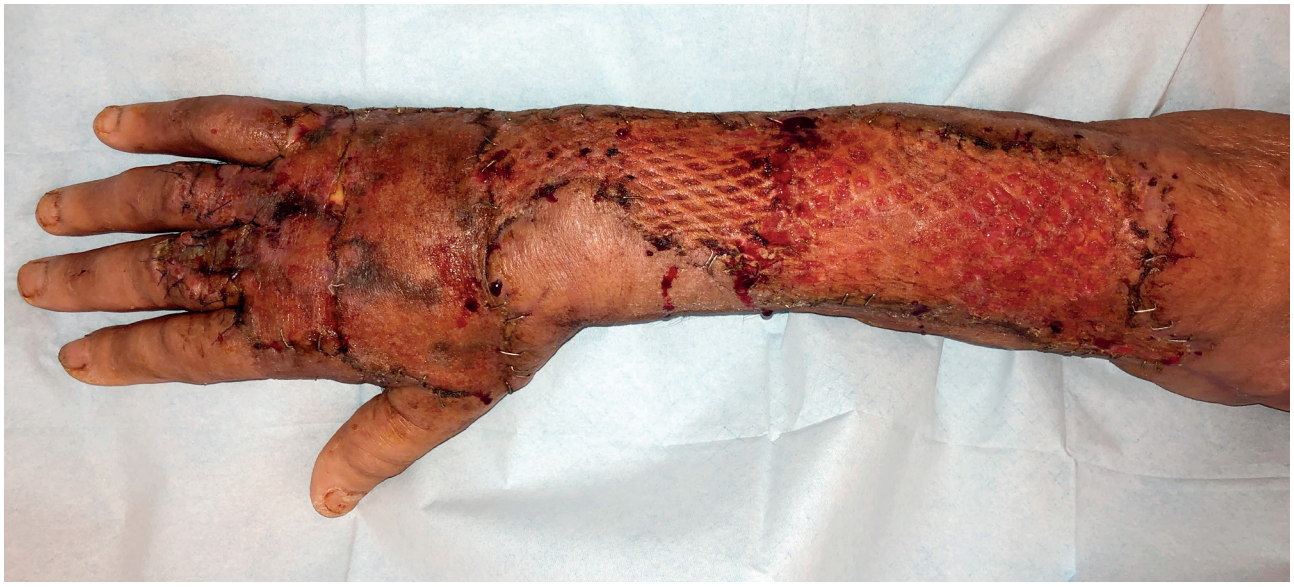

Figure 4 – Two months post-op

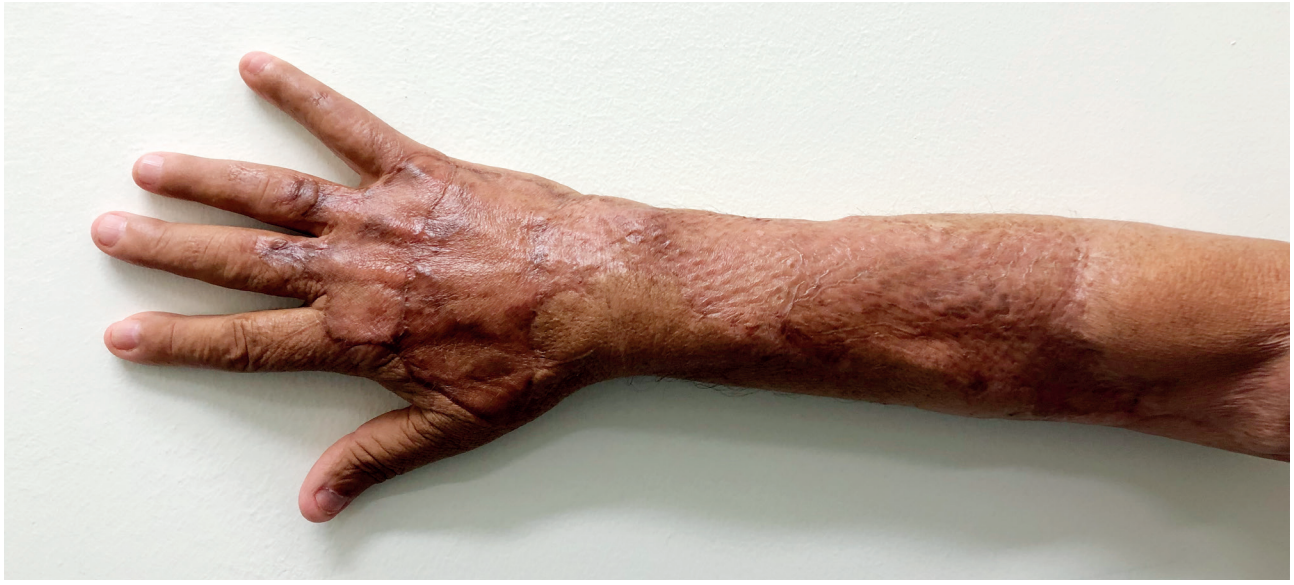

Figure 5 – Six months post-op

causative agent of a human infectious case,[14] posteriorly identified as *P. damselae.* A review of the soft tissue infections caused by this agent was made by Hundenborn *et al*[15] in 2013, reporting 11 infection cases. In the 11 cases reported, eight (73%) had a fatal outcome. In the three patients who could be cured, one required an amputation of the arm. When a search with the terms 'photobacterium damselae OR vibrio damselae' was carried out on the PubMed database and only case reports were selected, we retrieved 27 results, of which only 14 are skin or soft tissue infections in human. Most cases occurred in coastal areas of the United States of America, Australia, and Japan.

Other possible reconstructive options could be: a pedicle flap from another distal region (e.g. extended groin flap) with more complications expected; or a free flap (e.g. antero-lateral thigh) with important risks considering the A-V fistula in this arm, a possible blood steal syndrome in a patient on blood dialysis and anticoagulated. A skin graft without ADM was not possible because tendons did not have peritenon and there were no consistent local flaps available.

Necrotizing fasciitis is a rare infection that requires emergent surgery. A high degree of suspicious is necessary since delay in diagnosis can lead to loss of life or limb.

AUTHORS CONTRIBUTION

DG: Draft of the "Introduction" section.
LR: Draft of the "Case report" section.
LV: Draft of the "Discussion" section.
RC: Critical review of 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.

DATA CONFIDENTIALITY

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

INFORMED CONSENT

Obtained.

CONFLICTS OF INTEREST

All authors report no conflict of interest.

FUNDING SOURCES

The authors declare that there were no external sources of study for the performance of this article.

REFERENCES

1. Wong CH, Chang HC, Pasupathy S, Khin LW, Tan JL, Low CO. Necrotizing fasciitis: clinical presentation, microbiology, and determinants of mortality. J Bone Joint Surg Am. 2003;85:1454–60.
2. Love M, Teebken-Fisher D, Hose JE, Farmer JJ, Hickman FW, Fanning GR. Vibrio damsela, a marine bacterium, causes skin ulcers on the damselfish chromis punctipinnis. Science. 1981;214:1139–40.
3. Childers BJ, Potyondy LD, Nachreiner R, Rogers FR, Childers ER, Oberg KC, et al. Necrotizing fasciitis: a fourteen-year retrospective study of 163 consecutive patients. Am Surg. 2002;68:109–16.
4. Koshy JC, Bell B. Hand infections. J Hand Surg Am. 2019;44:46–54.
5. Chan T, Yaghoubian A, Rosing D, Kaji A, de Virgilio C. Low sensitivity of physical examination findings in necrotizing soft tissue infection is improved with laboratory values: a prospective study. Am J Surg. 2008;196:926–30.
6. Gonzalez MH. Necrotizing fasciitis and gangrene of the upper extremity. Hand Clin. 1998;14:635–45.
7. Wong CH, Khin LW, Heng KS, Tan KC, Low CO. The LRINEC (Laboratory Risk Indicator for Necrotizing Fasciitis) score: a tool for distinguishing necrotizing fasciitis from other soft tissue infections. Crit Care Med. 2004;32:1535–41.
8. Tsai YH, Hsu RW, Huang KC, Huang TJ. Laboratory indicators for early detection and surgical treatment of vibrio necrotizing fasciitis. Clin Orthop Relat Res. 2010;468:2230–7.
9. Chauhan A, Wigton MD, Palmer BA. Necrotizing fasciitis. J Hand Surg Am. 2014;39:1598–601.
10. Angoules AG, Kontakis G, Drakoulakis E, Vrentzos G, Granick MS, Giannoudis PV. Necrotising fasciitis of upper and lower limb: a systematic review. Injury. 2007;38:S18–25.
11. Elliott DC, Kufera JA, Myers RA. Necrotizing soft tissue infections. Risk factors for mortality and strategies for management. Ann Surg. 1996;224:672–83.
12. Stevens DL, Bisno AL, Chambers HF, Dellinger EP, Goldstein EJ, Gorbach SL, et al. Executive summary: practice guidelines for the diagnosis and management of skin and soft tissue infections: 2014 update by the Infectious Diseases Society of America. Clin Infect Dis. 2014;59:147–59.
13. Fontes RA, Ogilvie CM, Miclau T. Necrotizing soft-tissue infections. J Am Acad Orthop Surg. 2000;8:151–8.
14. Morris JG, Miller HG, Wilson R, Tacket CO, Hollis DG, Hickman FW, et al. Illness caused by vibrio damsela and vibrio hollisae. Lancet. 1982;1:1294–7.
15. Hundenborn J, Thurig S, Kommerell M, Haag H, Nolte O. Severe wound infection with photobacterium damselae ssp. damselae and vibrio harveyi, following a laceration injury in marine environment: a case report and review of the literature. Case Rep Med. 2013;2013:610632.

Doença de Creutzfeldt-Jakob: Apresentação Atípica de uma Doença Muito Rara

Creutzfeldt-Jakob Disease: Atypical Presentation of a Very Rare Disease

CASO CLÍNICO

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RESUMO

A doença de Creutzfeldt-Jakob, manifesta-se habitualmente como demência rapidamente progressiva. Apresentamos um caso de um doente de 59 anos com quadro súbito de parésia facial central e disartria, seguindo-se mioclonias no hemicorpo esquerdo. A ressonância magnética crânio-encefálica inicial mostrava hipersinal T2 e difusão no caudado e putamen direitos e líquido cérebro-raquidiano com hiperproteinorraquia. A investigação para causas autoimunes, infecciosas e paraneoplásicas foi negativa. Verificou-se um agravamento progressivo nos meses seguintes para hemiplegia esquerda, disfagia, incontinência urinaria e posterior mutismo acinético. A ressonância magnética crânio-encefálica mostrou evolução para restrição à difusão dos gânglios da base bilateralmente e múltiplas áreas corticais; A eletroencefalografia mostrou atividade periódica e proteína Tau no líquido cérebro-raquidiano elevada. A análise genética revelou mutação c.598G > A. O falecimento ocorreu após quatro meses de doença. Reportamos um caso de doença de Creutzfeldt-Jakob familiar associada a mutação da proteína priónica, com apresentação clínica e radiológica atípicas, nomeadamente sinais focais com instalação súbita, ausência de defeito cognitivo significativo e alterações imagiológicas unilaterais. Na evolução, a clínica e imagem tornaram-se características, permitindo o diagnóstico.

Palavras-chave: Mutação/genética; Priões/genética; Síndrome de Creutzfeldt-Jakob/diagnóstico; Síndrome de Creutzfeldt-Jakob/genética

ABSTRACT

Creutzfeldt-Jakob disease typically presents as rapidly progressive dementia. We describe the case of a 59-year-old male patient presenting with sudden onset of central facial palsy and dysarthria, followed by myoclonus of his left upper and lower limbs. Initial brain magnetic resonance showed hyperintensity of the right caudate and putamen on diffusion-weighted imaging and T2 sequences. Cerebrospinal fluid analysis showed increased protein count. The workup to investigate autoimmune, infectious and paraneoplastic causes was negative. Symptoms progressively worsened, with left hemiplegia, dysphagia, urinary incontinence, and, later, akinetic mutism. The follow-up brain magnetic resonance scan revealed hyperintensity of bilateral basal ganglia as well as cerebral cortical abnormalities on diffusion-weighted imaging. Electroencephalography showed periodic activity and tau protein levels in the cerebrospinal fluid were elevated. Genetic analysis showed mutation c-598G > A. The patient died four months later. We report a case of familial Creutzfeldt-Jakob disease with atypical clinical and radiological features, namely neurological focal signs with sudden onset, absence of significant cognitive impairment and unilateral radiological findings. With disease progression, characteristic clinical and radiological features led to the diagnosis.

Keywords: Creutzfeldt-Jakob Syndrome/diagnosis; Creutzfeldt-Jakob Syndrome/genetics; Mutation/genetics; Prions/genetics

INTRODUÇÃO

As doenças priónicas, ou encefalopatias espongiformes transmissíveis, são doenças degenerativas que afetam o sistema nervoso central de animais e humanos[1] (Tabela 1). A proteína priónica normal (PrPC), presente em todas as espécies mamíferas, é codificada pelo gene priónico (PRNP) no cromossoma 20,[1–3] desconhecendo-se o seu papel fisiológico. Nestas doenças a proteína priónica é convertida numa forma anormal denominada prião.[1–3]

A doença de Creutzfeldt-Jakob (DCJ),forma mais comum de doença priónica humana, é muito rara, com taxa de incidência anual de 1 - 2 casos/milhão a nível global.[3] A forma esporádica (sDCJ) corresponde a 80% - 95% dos casos.[2] Estima-se que 10% a 15% dos casos estejam associados a mutações de PRNP (DCJ familiar/hereditária) e que 1% sejam iatrogénicos, e relacionados com transplantes de dura-máter ou córnea, e com o uso de instrumentos neurocirúrgicos contaminados.[2] Existe ainda a forma variante relacionada com o consumo de produtos contaminados, atualmente rara.[2]

A sDCJ tem pico de incidência na sétima década de vida, e caracteriza-se por alteração cognitiva rapidamente progressiva associada a sinais neurológicos, mais frequentemente mioclonias e disfunção cerebelosa,[4] com sobrevida média de seis meses.[2] O diagnóstico definitivo consiste na identificação de priões em tecido cerebral *post mortem*. Contudo, é possível fazer o diagnóstico utilizando critérios do Centers for Disease Control and Prevention (CDC), que consideram como critérios alterações no eletroencefalograma (EEG), ressonância magnética crânio-encefálica (RM-CE), e marcadores no líquido cefalorraquidiano (LCR) (Fig. 1).

A DCJ familiar é semelhante à forma esporádica, apre-

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Tabela 1 – Encefalopatias espongiformes transmissíveis - Doenças Priónicas Humanas e seus acrónimos, e correspondentes doenças priónicas animais.

Doenças humanas priónicas	Encefalopatias espongiformes transmissíveis animais
DCJ esporádica DCJ iatrogénica DCJ familiar ou genética DCJ variante	Tremor epizoótico Tremor epizoótico atípico
Síndrome de Gerstmann-Straussler-Scheinker	Encefalopatia espongiforme dos visons
Insónia fatal esporádica Insónia fatal familiar	Doença crónica emaciante
Prionopatia variavelmente sensível à protease	Encefalopatia espongiforme bovina
Kuru Angiopatia amilóide cerebral de proteína priónica	Encefalopatia espongiforme dos felinos

DCJ; doença de Creutzfeldt-Jakob

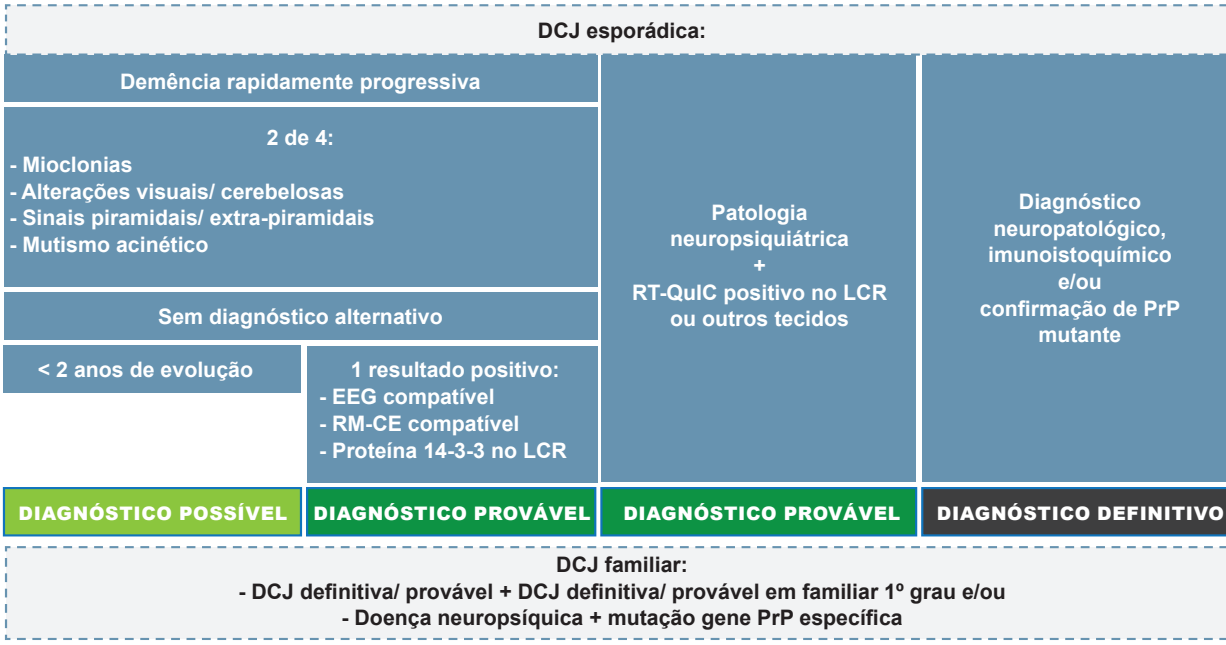

Figura 1 – Critérios do Centers for Disease Control and Prevention para o diagnóstico de doença de Creutzfeldt-Jakob (DCJ)
DCJ: doença de Creutzfeldt-Jakob; EEG: eletroencefalograma; LCR: líquido cérebro-raquidiano; PrP: proteína priónica; RM-CE: ressonância magnética crânio-encefálica; RT-QuIC: *real-time quaking-induced conversion*

sentando-se em idades mais precoces (40 - 50 anos) e com sobrevida ligeiramente maior (até dois anos).[2] A mutação E200K é a mais comum a nível global.[2]

Apresentamos um caso de DCJ definitiva em que a marcha diagnóstica foi desafiadora pela apresentação clínica e radiológica atípica.

CASO CLÍNICO

Doente do sexo masculino de 59 anos com antecedentes de hemocromatose, acidente isquémico transitório e glaucoma. Apresentava história familiar em dois tios paternos de morte aos 60 anos por doença neurológica não especificada.

Apresentou-se com disartria e parésia facial de instalação súbita, com melhoria em poucos dias. Após uma semana iniciou movimentos involuntários do membro superior esquerdo (MSE), associados a disartria, ligeira anomia (dificuldade na nomeação de pessoas e objetos) e discalculia (dificuldade no cálculo) Não apresentava febre ou outra sintomatologia. Ao exame neurológico não apresentava alteração significativa das funções nervosas superiores, com disartria ligeira, parésia facial central direita, movimentos mioclónicos do MSE e hemiface esquerda (Tabela 2).

A RM-CE (Fig. 2A) mostrou alteração em T2 e difusão (DWI) no caudado e putamen direitos, sem envolvimento do estriado contralateral. Por manter agravamento, repetiu RM-CE (Fig. 2B) após cinco dias, tendo-se verificado adicional hipersinal em difusão do córtex fronto-parieto-cingular medial. A investigação realizada para identificação de causas autoimunes, infecciosas e paraneoplásica resultou negativa, LCR apresentava aumento da proteína tau e proteína 14.3.3 normal. O EEG revelou atividade lenta parietal direita e frontotemporal bilateral (Tabela 2). Nas duas semanas seguintes, verificou-se agravamento da disartria

Tabela 2 – Resumo das características clínicas, laboratoriais e radiológicas ao longo do curso da doença

Sexo/ Idade	♂, 59 anos		
Tempo de sintomas até ao diagnóstico	1 mês		
Clínica inicial/ evolutiva	Disartria Parésia facial central direita Mioclonias* Discalculia	Hemiplegia esquerda Disfagia	Incontinência urinaria Mutismo acinético
Laboratorialmente	Proteína Tau > 1700 Proteína 14.3.3 negativa		
Diagnóstico diferencial	Estudo **negativo** para causas autoimunes, infeciosas, paraneoplásicas, incluindo: - VS, PCR, autoimunidade sistémica (ANA, SSA/B, ANCAs, SAF, anti-Tiroideus), Anti-Hu, e anticorpos associados a encefalites auto-imunes (NMDA, LGI1, AMPA, GABA, CASPR2); - LCR: bandas oligoclonais, exames culturais, exame citológico, pesquisa de vírus neurotrópicos; - TC tóraco-abdomino-pélvica		
Evolução radiológica (restrição à difusão - RM-CE)	Núcleo caudado e putamen direito	Córtex fronto-pareito-cingular medial e pericentral à direita	Gânglios da base bilateralmente e múltiplas áreas corticais
Evolução eletroencefalográfica (EEG)	Atividade lenta difusa	Atividade periódica complexos ponta-onda	
Tempo de sintomas até à morte	4 meses		

EEG: eletroencefalograma; LCR: líquido-céfalo-raquidiano; RM-CE: ressonância magnética crânio-encefálica; ♂: sexo masculino; *Mioclonias: movimentos mioclónicos do membro superior esquerdo desencadeados pela extensão do punho e mioclonias rítmicas da hemiface esquerda com expansão aos músculos da região cervical ipsilateral desencadeados pela contração voluntária dos músculos da hemiface esquerda.

e surgimento de ataxia da marcha. Colocou-se a possibilidade de encefalite autoimune, tendo sido administrada metilprednisolona endovenosa (1 g durante cinco dias), sem melhoria.

Nos meses seguintes a deterioração neurológica manteve-se progressiva, com evolução para anartria (incapacidade de articulação verbal) e disfagia, hemiparesia esquerda, incontinência urinária e agravamento cognitivo com alteração da memória recente, desorientação temporal e disfunção executiva. Foi realizada nova RM-CE (Fig. 2C), que mostrou restrição à difusão dos gânglios da base bilateralmente e múltiplas áreas corticais, predominantemente córtex frontal e parietal. O EEG identificou atividade periódica de complexos ponta-onda generalizadas com reatividade à estimulação. O doente viria a falecer aos quatro meses de doença.

A análise genética efetuada posteriormente resultou positiva para a mutação c.598G > A (E200K).

DISCUSSÃO

Na prática clínica, a DCJ surge mais frequentemente no diagnóstico diferencial de demência rapidamente progressiva. Nalguns casos, pode mimetizar outras doenças neurológicas ou psiquiátricas e os exames complementares iniciais podem não apresentar alterações características. Estima-se que muitos doentes já ultrapassaram dois terços do curso da doença aquando do diagnóstico.[3]

Os sintomas cognitivos são a forma mais comum de apresentação, seguidos em igual frequência por sintomas cerebelosos, constitucionais e comportamentais (20% cada).[2] Sintomas de disfunção cortical (por exemplo, afasia, apraxia, *neglect*) ocorrem precocemente em 15% dos casos, e em metade dos casos durante a evolução da doença.[2] O fenótipo clássico caracteriza-se por demência rapidamente progressiva associada a ataxia e mioclonias. No caso que relatamos, apenas as mioclonias fizeram parte da apresentação clínica inicial, que consistiu em sinais neurológicos focais de instalação súbita, sem defeito cognitivo significativo. Estão descritas outras apresentações atípicas, incluindo *alien-limb*, parésias isoladas de pares cranianos, e variantes clínicas como as variantes visual e cerebelosa.[1,2,4]

Até ao momento existem cerca de 50 mutações patogénicas do gene *PRNP*, sendo a *E200K* a mais prevalente.[5] Embora as alterações dos exames complementares sejam semelhantes à sDCJ, existem algumas diferenças clínicas. Crises convulsivas, cefaleia, neuropatia periférica, parésia da supraversão do olhar e alterações do sono (predominantemente insónia) são mais frequentemente reportadas em doentes com mutação *E200K*.[5,6]

Em Portugal, a maioria dos casos reportados são de sDCJ. Numa das maiores séries de casos, no norte de Portugal, apenas dois dos 11 doentes incluídos tinham história familiar de demência, e apenas um cumpria critérios de DCJ familiar. Não se identificaram mutações PRNP.[7]

Comparativamente à sDCJ, em que a deteção de proteína 14-3-3 no LCR apresenta alta sensibilidade (90% - 96%), a elevação deste e de outros biomarcadores do LCR é menos frequente nas formas de DCJ geneticamente determinadas,[2,8] o que vai ao encontro do caso clínico repor-

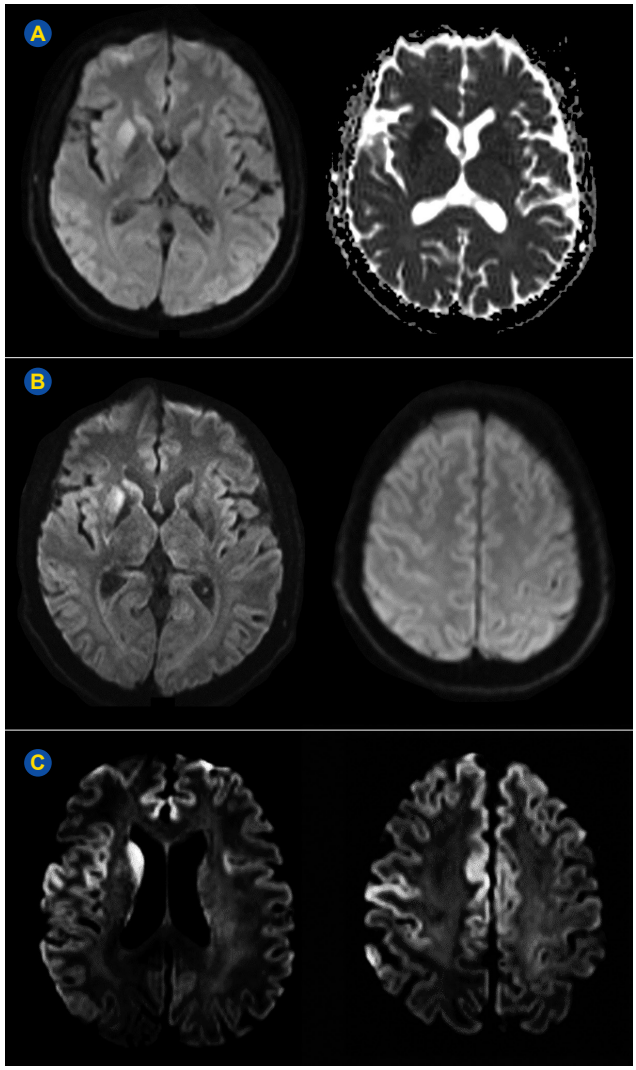

Figura 2 – Evolução radiológica. Evolução das lesões com restrição à difusão na RM-CE ao longo do curso da doença. (A) Alteração em T2 e difusão no caudado e putamen direito. Sem envolvimento do estriado contralateral; (B) Restrição à difusão no caudado e putamen direito, com discreto hipersinal em difusão do córtex fronto-parieto-cingular medial e pericentral a direita. (C) Restrição à difusão dos gânglios da base bilateralmente e múltiplas áreas corticais; RM-CE – ressonância magnética crânio-encefálica.

tado, em que não se detetou proteína 14-3-3 no LCR.

A RM-CE é considerada o exame complementar mais útil na investigação, com sensibilidade global de 92,3%.[9] As alterações clássicas incluem hipersinal do caudado, putamen ou córtex (ou combinação entre estes) especialmente nas sequências de difusão e FLAIR, mas também em T2. No caso apresentado, a RM-CE inicial mostrou hipersinal em T2 e difusão no caudado e putamen apenas à direita. A maioria dos estudos longitudinais mostra que usualmente as alterações do putamen e caudado são bilaterais e simétricas, apesar de inicialmente o hipersinal envolver apenas parte do putamen. Alguns estudos reportam lesões unilaterais em DWI,[6,9] numa fase precoce da doença, mas são anedóticos os casos com lesões unilaterais em T2.[10]

Na prática clínica deve ser tida em consideração a possibilidade de apresentações atípicas de DCJ, sendo importante nestes casos a exclusão dos diagnósticos diferenciais. Exames complementares mais sensíveis e específicos, como as recentes técnicas de *real-time quaking-induced conversion* (RT-QuIC) que permitem detetar quantidades pequenas de proteína priónica no LCR e até no epitélio nasal[11,12], podem permitir um diagnóstico confirmatório mais precoce.

CONTRIBUTO DOS AUTORES

RO: Conceptualização, redação e revisão.
MD: Conceptualização e revisão.
IBM: Redação e revisão.

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.

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.

FONTES DE FINANCIAMENTO

Não foi utilizada nenhuma bolsa ou subsídio para a realização do trabalho.

REFERÊNCIAS

1. Head MW, Ironside JW. Review: Creutzfeldt-Jakob disease: prion protein type, disease phenotype and agent strain: CJD and prion agent strain. Neuropathol Appl Neurobiol. 2012;38:296-310.
2. Geschwind MD. Prion diseases. Continuum. 2015;21:1612-38.
3. Kojima G, Tatsuno BK, Inaba M, Velligas S, Masaki K, Liow KK. Creutzfeldt-Jakob disease: a case report and differential diagnoses. Hawaii J Med Public Health J Asia Pac Med Public Health. 2013;72:136-9.
4. Takada LT, Kim MO, Cleveland RW, Wong K, Forner S, Gala I, et al. Genetic prion disease: experience of a rapidly progressive dementia center in the United States and a review of the literature. Am J Med Genet B Neuropsychiatr Genet. 2017;174:36-69.
5. Gao LP, Shi Q, Xiao K, Wang J, Zhou W, Chen C, et al. The genetic Creutzfeldt-Jakob disease with E200K mutation: analysis of clinical, genetic and laboratory features of 30 Chinese patients. Sci Rep. 2019;9:1836.
6. Ukisu R, Kushihashi T, Kitanosono T, Fujisawa H, Takenaka H, Ohgiya Y, et al. Serial diffusion-weighted MRI of Creutzfeldt-Jakob disease. Am J Roentgenol. 2005;184:560-6.
7. Silva AM, Pires MM, Leite AJ, Honavar M, Mendes A, Correia M, et al. Estudo retrospectivo da doença de Creutzfeldt-Jakob diagnosticada no norte de Portugal entre 1993-2002: características demográficas, clínicas e neuropatológicas. Arq Neuropsiquiatr. 2003;61:950-6.
8. Collins S, Boyd A, Fletcher A, Gonzales M, McLean C, Byron K, et

al. Creutzfeldt-Jakob disease: diagnostic utility of 14–3–3 protein immunodetection in cerebrospinal fluid. J Clin Neurosci. 2000;7:203-8.
9. Collie DA, Sellar RJ, Zeidler M, Colchester AC, Knight R, Will RG. MRI of Creutzfeldt–Jakob disease: imaging features and recommended MRI protocol. Clin Radiol. 2001;56:726-39.
10. Murata T, Shiga Y, Higano S, Takahashi S, Mugikura S. Conspicuity and evolution of lesions in Creutzfeldt-Jakob disease at diffusion-weighted imaging. AJNR Am J Neuroradiol. 2002;23:1164-72.
11. Green AJ. RT-QuIC: a new test for sporadic CJD. Pract Neurol. 2019;19:49-55.
12. Orrú CD, Bongianni M, Tonoli G, Ferrari S, Hughson A, Groveman B, et al. A test for Creutzfeldt–Jakob disease using nasal brushings. N Engl J Med. 2014;371:519-29.

A Shattered Ulna

Uma Ulna Estilhaçada

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Keywords: Forearm Injuries; Fractures, Comminuted; Ulna/injuries; Ulna Fractures; Wounds, Gunshot
Palavras-chave: Lesões do Antebraço; Lesão por Arma de Fogo; Fraturas Cominutivas; Fraturas da Ulna; Ulna/lesões

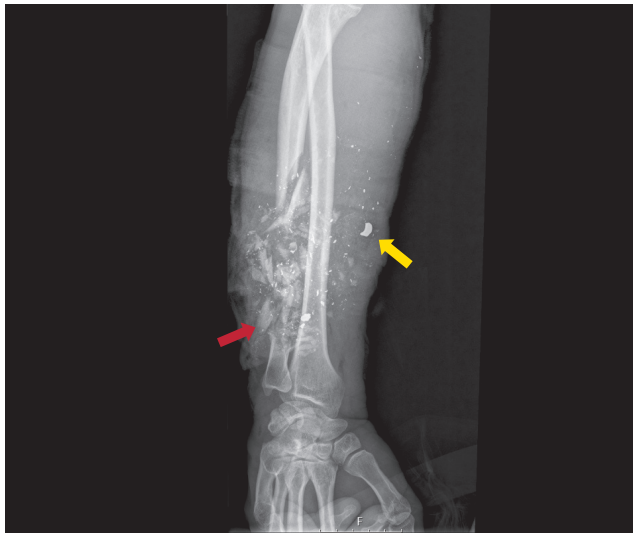

Figure 1 – Radiograph of the right forearm, at admission. Yellow arrow: metallic fragment; red arrow: bone fragment.

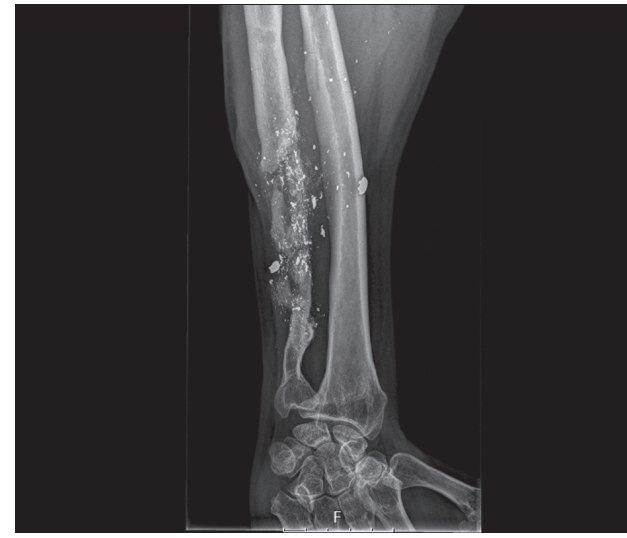

Figure 2 – Radiograph of the right forearm, two years after the injury

A 41-year-old man was admitted to the emergency department with severe gunshot injuries: three penetrating wounds at the volar side and ulnar border of the right forearm, with bone exposure. The radiograph showed an irregular multi-fragmented meta-diaphyseal fracture of the ulna with multiple small metal fragments spread across the forearm (Fig. 1). External fixation was used for stabilization during five months. Two years later, the radiograph showed nonunion of the remaining fragments, that were insufficiently bridged by mature bone (Fig. 2).

Gunshot fractures of the ulna are infrequent and there is scarce literature about their management.[1] Severe comminution may arise without high local energy transfer, due either to very fast transfer or to concentration in a small area.[2-4] Although gunshot injuries often result in neurovascular lesion or infection,[1] a highly-comminuted gunshot fracture might have a relatively maintained soft-tissue envelope, which must be preserved with an appropriate stabilization method to allow for consolidation.[2]

AUTHORS CONTRIBUTION

FV: Conception and coordination of the work; draft of the manuscript.

FM: Analysis and description of the images; draft of the manuscript.

EP: Draft of the manuscript; critical review.

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REFERENCES

1. Mehta SK, Dale WW, Dedwylder MD, Bergin PF, Spitler CA. Rates of neurovascular injury, compartment syndrome, and early infection in operatively treated civilian ballistic forearm fractures. Injury. 2018;49:2244-7.
2. Bowyer GW, Rossiter ND. Management of gunshot wounds of the limbs. J Bone Joint Surg Br. 1997;79:1031-6.
3. Wilson AJ. Gunshot injuries: what does a radiologist need to know? Radiographics. 1999;19:1358-68.
4. Hinsley DE, Phillips SL, Clasper JS. Ballistic fractures during the 2003 Gulf conflict--early prognosis and high complication rate. J R Army Med Corps. 2006;152:96-101.

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Trombose, Hemorragia e Trombocitopenia Induzidas pelas Vacinas contra a COVID-19: Protocolo de Atuação

Guidelines on COVID-19 Vaccine Induced Thrombosis, Bleeding, and Thrombocytopenia

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RESUMO

Após a generalização da vacinação contra a COVID-19, foram relatados efeitos adversos como trombose, hemorragia e trombocitopenia. Recentemente, após vacinação, foi reconhecido um síndrome raro e com mortalidade elevada, caraterizado por uma combinação não usual de trombocitopenia e trombose, em particular trombose dos seios venosos cerebrais, com muitas semelhanças com a trombocitopenia induzida pela heparina. Foram desenvolvidas diferentes recomendações na definição, diagnóstico e tratamento destas raras complicações. Apresentamos aqui, um protocolo de atuação baseado na evidência atual.
Palavras-chave: Vacinas Anti-COVID-19; Hemorragia; Trombocitopenia; Trombose

ABSTRACT

After widespread vaccination with COVID-19 vaccines, there have been worldwide reports on thrombosis, bleeding, and thrombocytopenia. Recently, a rare syndrome with a high mortality rate consisting of an unusual combination of thrombocytopenia and thrombosis, in particular cerebral venous sinus thrombosis, which clinically resembles heparin-induced thrombocytopenia, was reported following vaccination. Different statements and recommendations were developed regarding the definition, diagnosis, and treatment of these rare complications. We present here a protocol with recommendations, based on current evidence.
Keywords: COVID-19 Vaccines; Hemorrhage; Thrombocytopenia; Thrombosis

INTRODUÇÃO

A infeção pelo SARS-CoV-2 (COVID-19), que se associa a considerável morbilidade e mortalidade, tornou-se rapidamente numa pandemia global com impacto na saúde das populações e na economia de cada país. Tal como para outras infeções, a vacinação parece ser a principal forma de controlo da doença, pelo que desde cedo foi grande o esforço no sentido do desenvolvimento de vacinas, sendo que algumas destas já se encontram licenciadas e em uso generalizado.

Foi precisamente a generalização da vacinação que fez emergir novos efeitos adversos, e o aparecimento dos primeiros relatos de associação das vacinas baseadas no RNA mensageiro (RNAm) com trombocitopenia grave e hemorragia, mas sem trombose.[1] Logo no início de janeiro, foi relatado nos EUA o caso de um profissional de saúde que, 16 dias após inoculação com a vacina produzida pela Pfizer-BioNTech, apresentou uma hemorragia fatal associada a trombocitopenia imune.[1] Seguiu-se imediatamente a identificação de vários casos de trombocitopenia, com resposta favorável a terapias dirigidas à trombocitopenia imune [corticóides e imunoglobulina humana IV (IgIV)].[2] Atualmente, os casos de 'trombocitopenia' ou 'trombocitopenia imune'(PTI), com ou sem associação a hemorragia e mortalidade, são transversais às quatro vacinas aprovadas pela European Medicines Agency - EMA, e têm sido reportados quer na *Vaccine Adverse Event Reporting System* (VAERS) nos Estados Unidos, quer na *EudraVigilance* na Europa, quer no *MHRA Yellow Card* no Reino Unido, entre outros. Aliás a PTI é uma complicação já bem conhecida, embora rara, de várias outras vacinas.[3]

No início de março de 2021, na Europa, começaram a surgir preocupações sobre o aparecimento de eventos trombóticos não usuais na sequência da vacinação com a vacina ChAdOx1 nCoV-19 da AstraZeneca (Vaxzevria), seguidas da declaração pela EMA, a 18 de março, garantindo que o número de eventos observados não seria superior ao esperado e aconselhando a continuação da vacinação com a Vaxzevria, uma vez que os benefícios superariam os riscos.[4] Pouco tempo depois, três grupos independentes, da Noruega,[5] Alemanha/Áustria,[6] e Reino Unido,[7] descreveram um total de 39 pessoas com um novo síndrome caracterizado por trombose, trombocitopenia e presença de anticorpos circulantes contra o fator plaquetário 4 (FP4).[8] Estes doentes, 66,7% mulheres e média etária de 42,5 anos (a variar entre 21 e 77 anos), foram admitidos no hospital cinco a 24 dias após vacinação, com tromboses atípicas em localização não usual, com predomínio das tromboses dos seios venosos cerebrais (TSVC) em dois terços dos casos e tromboses esplâncnicas. Tromboses típicas como trombose venosa profunda (TVP) ou embolia pulmonar (EP) assim como eventos arteriais, também foram descritos.[5-8] Dois destes doentes apresentaram trombocitopenia sem trombose (um deles faleceu com uma hemorragia cerebral sem que uma TSVC pudesse ter sido excluída,[6]

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e um outro apresentou apenas sintomas hemorrágicos[7]). A mortalidade foi superior à esperada (aproximadamente 40%) como consequência da lesão isquémica cerebral e/ou hemorragia cerebral.[9] O número de plaquetas ao diagnóstico variou entre cerca de 10 000 e 110 000/mm^3, e os níveis de D-dímeros apresentavam-se acentuadamente aumentados acompanhados por níveis baixos a normais de fibrinogénio.[8] Em quase todos os doentes foram identificados níveis elevados de anticorpos anti-FP4, detetados por testes de ELISA usados na suspeita de trombocitopenia induzida pela heparina (TIH), mas outros testes usados por rotina (quimioluminiscência ou imunoensaios por látex) são frequentemente negativos.[8]

Esta combinação não usual de trombocitopenia e trombose, em particular TSVC, que partilha muitas semelhanças com a TIH, é uma condição altamente pró-trombótica e com mortalidade elevada, tendo sido proposta a designação de *Vaccine-induced Immune Thrombocytopenia and Thrombosis* ou *Vaccine-induced Immune Thrombotic Thrombocytopenia* (VITT).[8] Ao contrário da TIH, na VITT a formação dos anticorpos anti-FP4 ocorre na ausência de exposição à heparina, mimetizando uma entidade previamente descrita[10] denominada TIH autoimune ou atípica, na qual a trombose surge na ausência de exposição prévia conhecida à heparina.

Mais recentemente, surgiu a publicação de um caso de VITT 14 dias após vacinação com a vacina Ad26.COV2.S da Johnson & Johnson/Janssen (vJ&J),[11] seguido pelo relato de 12 outros casos com o mesmo fenótipo clínico e laboratorial dos casos descritos após vacinação com a Vaxzevria.[12]

São vários os casos revistos pelo Comité de Segurança da EMA associados à Vaxzevria (em 4 de abril, um total de 169 casos de TSVC e 53 casos de tromboses venosas esplâncnicas).[13] Muitos outros têm sido reportados associados à vacinação com as vacinas RNAm (Pfizer- BioNTech e Moderna), embora nem todos tenham sido revistos centralmente ou haja informação sobre a avaliação dos anticorpos anti-FP4.[9] A taxa de incidência estimada de VITT é de cerca de 1 caso por 100 000 vacinações,[9] a variar entre 1 caso por 26 500 e 1 caso por 127 300 vacinações.[14] Pensa-se, no entanto, que a taxa de incidência esteja subestimada, devido à não obrigatoriedade da notificação.

RECOMENDAÇÕES

O reconhecimento desta nova entidade, levou ao rápido aparecimento de protocolos de diagnóstico e orientação clínica de diferentes sociedades científicas nacionais e internacionais (Fig. 1).[15-21] Da síntese dessas orientações, sugerimos as seguintes recomendações:

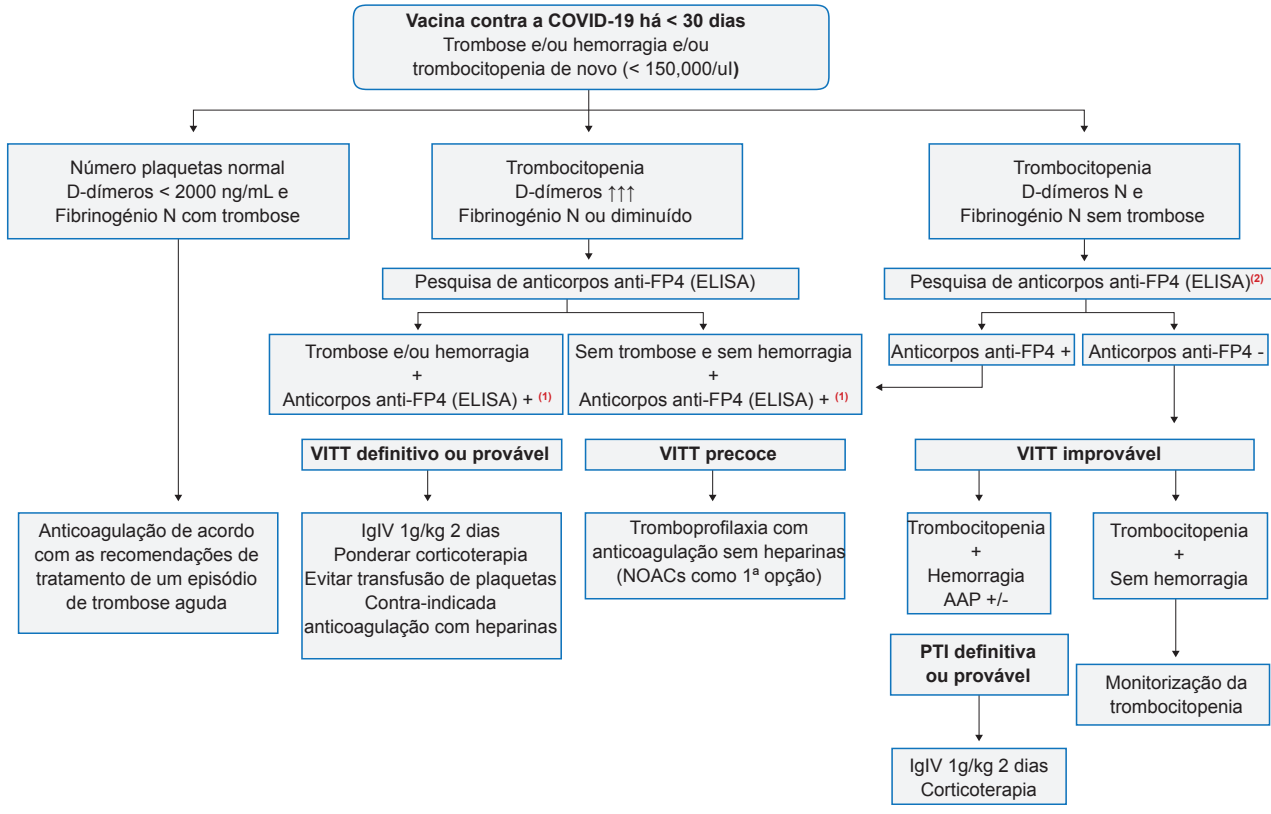

Figura 1 – Estratégias de diagnóstico e tratamento da trombocitopenia, trombose e/ou hemorragia, associada à vacinação contra a COVID-19

VITT: *vaccine-induced Immune Thrombocytopenia and Thrombosis*; PTI: púrpura trombocitopénica imune; FP4: fator plaquetário 4; IgIV: imunoglobinas intra-venosas; AAP: anticorpos antiplaquetários.

(1): na ausência de resultado de anticorpos anti-FP4, assumir VITT provável.

(2): não obrigatória pesquisa de anti-corpos anti-FP4, nesta situação.

1. Até 30 dias após vacinação, em particular com as vacinas Vaxzevria e vJ&J, deve ser procurada assistência médica imediata na presença de[13,15,8,20]:

- sintomas neurológicos, incluindo tonturas, dores de cabeça graves e persistentes ou visão turva;
- dor abdominal persistente;
- falta de ar ou dor no peito;
- edema ou dor persistente nos membros;
- presença de petéquias ou equimoses para além do local da injeção.

Sintomas semelhantes aos da gripe, como artralgias, mialgias ou cefaleias, que persistem por um a dois dias após a vacinação, são efeitos comuns e não são motivo de preocupação.[13, 15]

2. Em presença dos sintomas referidos, devem ser realizados estudos de imagem apropriados para diagnóstico de trombose aguda e avaliação analítica, que inclui hemograma completo com contagem de plaquetas e esfregaço de sangue periférico (para confirmação de trombocitopenia), e estudo de coagulação com D-dímeros e fibrinogénio.[15, 17-19]

3. Perante episódio de trombose aguda confirmado, com contagens de plaquetas normais, D-dímeros < 2000 ng/mL e fibrinogénio normal, é possível excluir a VITT. O tratamento é o tratamento anticoagulante de qualquer outro episódio agudo de trombose.

4. Em presença de trombocitopenia e evidência de trombose aguda e/ou hemorragia deve ser efetuada pesquisa de anticorpos anti-FP4[15-21]:

- Indicados testes por ELISA para deteção de TIH (baseados na deteção imunológica de anticorpos contra o complexo FP4/heparina).[15-19]
- Nem todos os testes comerciais para diagnóstico de TIH podem ser usados (não estão indicados testes por outros métodos, como Acustar ou o método latex).[15,16,19,21]
- Testes funcionais anti-FP4, se disponíveis, ajudam a diferenciar uma TIH autoimune de VITT,[15,19] embora a sua utilidade não seja muito clara.[12]
- Na ausência de disponibilidade imediata de um teste anti-FP4, a amostra de sangue periférico colhida antes do tratamento, deve ser congelada, para posterior deteção de anticorpos.[16]

5. Diagnóstico definitivo de VITT, se trombocitopenia, D-dímeros muito aumentados e trombose progressiva, com uma grande preponderância de TSVC; a hemorragia também pode ser significativa e inesperada.[16]

- Tipicamente contagem de plaquetas inferior a 150 x 10^9/L, níveis muito aumentados de D-dímeros (> 4000 ng/mL) e em alguns casos níveis baixos de fibrinogénio; foi reconhecido recentemente que até 5% dos doentes podem ter contagens de plaquetas normais na apresentação, podendo desenvolver trombocitopenia nos dias subsequentes.[16]
- Presença de anticorpos anti-FP4 (por testes ELISA) na ausência de exposição à heparina.

6. Diagnóstico provável de VITT, se trombose e/ou hemorragia, trombocitopenia e D-dímeros muito aumentados e níveis de fibrinogénio normais ou baixos. Na ausência de disponibilidade imediata para teste ELISA anti-FP4 tratar como VITT, enquanto se aguarda confirmação de diagnóstico.[16]

7. O tratamento do VITT definitivo ou provável passa por[15,16,19,21]:

- Tratamento imediato com IgIV, mesmo na ausência de confirmação do diagnóstico (1 g/kg/dia durante dois dias). Podem ser necessárias doses adicionais.
- Evitar transfusão de plaquetas. Se necessário procedimento neurocirúrgico, as plaquetas devem ser efetuadas durante ou após administrar IgIV.[16]
- Evitar qualquer administração de heparina (incluindo nos cateteres) pela possibilidade teórica de exacerbar a VITT.
- Anticoagulação com anticoagulantes sem heparina [fondaparinux, argatrobano - utilizado no tratamento da trombocitopenia induzida pela heparina em hospitais portugueses, por importação direta, e noutros hospitais europeus apesar de não estar aprovado pela EMA - danaparóide, anticoagulantes orais diretos (DOAC)] com doses ajustadas de acordo com a trombocitopenia[19]

  Se Plaquetas > 100x 10^9/L

  Fondaparinux: 5/7,5/10 mg para peso corporal < 50/50 – 100/ > 100 kg, respetivamente.

  Argatrobano: rácio de TTPA 1,5 – 2,5

  Se Plaquetas 50 - 100x 10^9/L

  Fondaparinux: 5/7,5 mg para peso corporal < 50 ou > 50 kg

  Argatrobano: rácio de TTPA 1,5 – 2,5

  Se Plaquetas 20 - 50x 10^9/L

  Fondaparinux: 2,5/5 mg para peso corporal < 50 ou > 50 kg

  Argatrobano: rácio de TTPA 1,5

  Se Plaquetas < 20 - 50x 10^9/L

  Evitar anticoagulação.

- Podem estar indicados corticoides, sobretudo se houver atraso na administração de IgIV.[16,19,20] Se o valor de plaquetas < 20x 10^9/L, está indicado associar dexametasona 40 mg/dia durante quatro dias[19] ou prednisolona 1 mg/kg/dia, se plaquetas < 50 x 10^9/L.[20]
- Considerar suplementação com concentrados de fibrinogénio, se níveis plasmáticos de fibrinogénio < 1,5 g/L.
- Considerar plasmaférese se se verificar deterioração clínica apesar do tratamento. No caso de trombose extensa, trombocitopenia < 30x 10^9/L ou níveis muito elevados de anticorpos anti-FP4, a plasmaférese deve ser considerada precocemente.[16]
- Não está recomendada antiagregação plaquetária.
- A presença de trombose complicada por hemorragia, em particular em doentes com TSVC, torna o tratamento um desafio. Não se deve introduzir anticoagulação enquanto houver hemorragia ativa.

- Em caso de trombose arterial, é preferida a anticoagulação sobre a antiagregação até à normalização dos valores de plaquetas, D-dímeros e fibrinogénio; só então é que se deve mudar para terapêuticas antiplaquetárias.[16]

8. Trombocitopenia sem trombose, com D-dímeros normais ou ligeiramente aumentados e fibrinogénio normal, torna o diagnóstico de VITT improvável.[16,21]

- Se trombocitopenia sem hemorragia, devem-se monitorizar continuamente os parâmetros clínicos e laboratoriais.[21]
- Trombocitopenia e hemorragia aponta para trombocitopenia imune (PTI), a confirmar pela presença de anticorpos antiplaquetários (AAP). Está indicado IgIV e/ou corticoides mas não anticoagulação.[21]

9. Trombocitopenia sem hemorragia ou trombose, mas com alterações nos parâmetros da coagulação (D-dímeros aumentados) pode indiciar uma VITT precoce, estando indicado iniciar tromboprofilaxia com anticoagulantes sem heparinas.[21]

10. História prévia de trombose ou trombofilia conhecida não são considerados fatores de risco para o desenvolvimento de VITT. Não há evidência de que tromboses em localizações típicas (TVP, EP) sejam mais comuns após vacinação do que na população em geral do mesmo grupo etário.[15]

11. Não está indicada profilaxia de rotina com anticoagulantes ou antiplaquetários na prevenção das tromboses atípicas associadas à vacinação.[15]

- Doentes a fazer anticoagulação oral por outras indicações [ex: fibrilação auricular ou tromboembolismo venoso (TEV)] devem continuar a anticoagulação durante e após vacinação.[15]
- Em doentes sem indicação para anticoagulação oral, mas em risco significativo de TEV (ex: sintomas gripais com febre e imobilização), pode estar indicada tromboprofilaxia farmacológica, avaliada numa base individual.[15]
- Se a tromboprofilaxia estiver indicada, considerar doses profiláticas de DOAC (rivaroxabano 10 mg/dia ou apixabano 2,5 mg 12/12 horas). Não está recomendada profilaxia com heparinas de baixo peso molecular (HBPM).[15]

12. Todos os casos de VITT, trombose, hemorragia ou trombocitopenia, 30 dias após administração de vacinas anti COVID-19, devem ser reportados, independentemente da probabilidade de associação com a vacinação.[16]

CONTRIBUTO DOS AUTORES

Ambos os autores contribuíram de igual forma para a concepção do artigo, revisão da literatura e redação do manuscrito.

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

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REFERÊNCIAS

1. Lee EJ, Cines DB, Gernsheimer T, Kessler C, Michel M, Tarantino MD, et al. Thrombocytopenia following Pfizer and Moderna SARS-CoV-2 vaccination. Am J Hematol. 2021;96:534-7.
2. Merchant HA. CoViD vaccines and thrombotic events: EMA issued warning to patients and healthcare professionals. J Pharm Policy Pract. 2021;14:32.
3. Perricone C, Ceccarelli F, Nesher G, Borella E, Odeh Q, Conti F, et al. Immune thrombocytopenic purpura (ITP) associated with vaccinations: a review of reported cases. Immunol Res. 2014;60:226-35.
4. European Medicines Agency. COVID-19 vaccine AstraZeneca: benefits still outweigh the risks despite possible link to rare blood clots with low blood platelets. March 18, 2021 [consultado 2021 mar 21]. Disponível em: https://www.ema.europa.eu/en/news/ covid-19-vaccine-astrazeneca-benefits-still-outweigh-risks-despite-possible-link-rare-blood-clots.
5. Schultz NH, Sørvoll IH, Michelsen AE, Munthe LA, Lund-Johansen F, Ahlen MT, et al. Thrombosis and thrombocytopenia after ChAdOx1 nCoV-19 vaccination. N Engl J Med. 2021;384:2124-30.
6. Greinacher A, Thiele T, Warkentin TE, Weisser K, Kyrle PA, Eichinger S. Thrombotic thrombocytopenia after ChAdOx1 nCov-19 vaccination. N Engl J Med. 2021;384:2092-101.
7. Scully M, Singh D, Lown R, Poles A, Solomon T, Levi M, et al. Pathologic antibodies to platelet factor 4 after ChAdOx1 nCoV-19 vaccination. N Engl J Med. 2021;384:2202-11.
8. Makris M, Pavord S, Lester W, Scully M, Hunt BJ. Vaccine-induced Immune Thrombocytopenia and Thrombosis (VITT). Res Pract Thromb Haemost. 2021;5:e12529.
9. Cines DB, Bussel JB. SARS-CoV-2 Vaccine–induced immune thrombotic thrombocytopenia. N Eng J Med. 2021; 384:2254-6.
10. Greinacher A, Selleng K, Warkentin TE. Autoimmune heparin-induced thrombocytopenia. J Thromb Haemost. 2017;15:2099-114.
11. Muir KL, Kallam A, Koepsell SA, Gundabolu K. Thrombotic thrombocytopenia after AD26.COV2.S vaccination. N Engl J Med 2021. 384:1964-5.
12. See I, Su JR, Lale A, Woo EJ, Guh AY, Shimabukuro TT, et al. US case reports of cerebral venous sinus thrombosis with thrombocytopenia after Ad26.COV2.S vaccination, March 2 to April 21, 2021. JAMA. 2021;e217517.
13. European Medicines Agency. AstraZeneca's COVID-19 vaccine: EMA finds possible link to very rare cases of unusual blood clots with low blood platelets. April 7, 2021. [consultado 2021 abr 10]. Disponível em: https://www.ema.europa.eu/en/news/astrazenecas -covid-19-vaccine-ema-finds-possible-link-very-rare-cases-unusual-blood-clots-low-blood.
14. Chan B, Odutayo A, Jüni P, Stall NM, Bobos P, Brown AD, et al. Risk of vaccine-induced thrombotic thrombocytopenia (VITT) following the AstraZeneca/COVISHIELD adenovirus vector COVID-19 vaccines. Science Briefs of the Ontario COVID-19 Science Advisory Table. Ontario: Ontario COVID-19 Science Advisory Table; 2021.
15. Oldenburg J, Klamroth R, Langer F, Albisetti M, von Auer C, Ay C, et al. Diagnosis and management of vaccine-related thrombosis following AstraZeneca COVID-19 vaccination: Guidance Statement from the GTH. Hamostaseologie. 2021 (in press). doi: 10.1055/a-1469-7481
16. British Society for Haematology. Guidance produced from the Expert Haematology Panel (EHP) focussed on Covid-19 Vaccine induced Thrombosis and Thrombocytopenia (VITT). Updated Guidance on Management. Version 2.0 28 May 2021 [consultado 2021 junho 16]. Disponível em: https://b-s-h.org.uk/media/19718/guidance-v20-20210528-002.pdf
17. Australian Government Department of Health. ATAGI statement on

AstraZeneca vaccine in response to new vaccine safety concerns. A statement from the Australian Technical Advisory Group on Immunisation (ATAGI) on the AstraZeneca COVID-19 vaccine in response to new vaccine safety concerns. April 8, 2021. [consultado 2021 abr 9]. Disponível em: https://www.health.gov.au/news/atagi-statement-on-astrazeneca-vaccine-in-response-to-new-vaccine-safety-concerns.

18. Thrombosis Canada. Vaccine-Induced Prothrombotic Immune Thrombocytopenia (VIPIT). 26 April 2021. [consultado 2021 abr 28]. Disponível em: https://thrombosiscanada.ca/wp-uploads/uploads/2021/04/51.-Vaccine-induced-prothrobotic-immune-thrombcytopenia_26Apr21-Final.pdf.
19. Gresele P, Marietta M, Ageno W, Marcucci R, Contino L, Donadini MP, et al. Management of cerebral and splanchnic vein thrombosis associated with thrombocytopenia in subjects previously vaccinated with Vaxzevria (AstraZeneca): a position statement from the Italian Society for the Study of Haemostasis and Thrombosis (SISET). Blood Transfus. 2021 (in press). doi: 10.2450/2021.0117-21.
20. International Society on Thrombosis and Haemostasis. ISTH Interim Guidance for the Diagnosis and Treatment on Vaccine Induced Immune Thrombotic Thrombocytopenia (Updated 20 April, 2021). [consultado 2021 abr 21]. Disponível em: https://cdn.ymaws.com/www.isth.org/resource/resmgr/ISTH_VITT_Guidance_2.pdf
21. Nazy I, Sachs UJ, Arnold DM, McKenzie SE, Choi P, Althaus K, et al. Recommendations for the clinical and laboratory diagnosis of vaccine-induced immune thrombotic thrombocytopenia (VITT) for SARS-CoV-2 infections: Communication from the ISTH SSC Subcommittee on Platelet Immunology. J Thromb Haemost. 2021;19:1585-8.

Resposta Imunológica à Primeira Dose da Vacina Moderna em Profissionais de Saúde com Infeção Prévia por SARS-CoV-2

Immune Response to Single-Dose Moderna Vaccination in Healthcare Workers Previously Infected with SARS-CoV-2

Palavras-chave: COVID-19/imunologia; Pandemia; Pessoal de Saúde; SARS-CoV-2; Vacinas COVID-19/imunologia
Keywords: COVID-19/immunology; COVID-19 Vaccines/immunology; Healthcare Workers; SARS-CoV-2; Pandemics

Caro Editor,

O Hospital-Escola da Universidade Fernando Pessoa (HE-UFP) recebeu as primeiras doses da vacina Moderna contra o SARS-Cov-2, em janeiro de 2021. No planeamento da administração da segunda dose da vacina, antecipámos que conseguiríamos oito doses a mais: quatro por melhor aproveitamento dos frascos em relação ao realizado no primeiro dia de vacinação com a primeira dose, e quatro profissionais que não poderiam receber a segunda dose (duas por gravidez e outros dois por infecção COVID-19).

Se por um lado podíamos usar estas doses para vacinar mais oito profissionais de saúde sem história de infecção COVID-19 prévia, por outro não tínhamos a garantia duma segunda inoculação no prazo estipulado pelo protocolo da Moderna (28 dias, de acordo com a Norma 01/2021 da Direção Geral da Saúde).[1] Assim, procedemos à vacinação de oito profissionais de saúde com história prévia de infecção COVID-19. Foram colhidas amostras serológicas (IgG) no próprio dia, que repetimos em D14 - 16 pós-inoculação. Os resultados são os apresentados na Tabela 1. Com exceção do profissional 1, todos tinham tido COVID-19 sintomático. Nenhum tinha tido necessidade de internamento e, à data de vacinação, encontravam-se assintomáticos. Foi obtido o consentimento informado dos profissionais para a inclusão no estudo.

Literatura recente tem mostrado que doentes previamente infectados com SARS-CoV-2 podem necessitar apenas de uma dose de vacina para desenvolver uma imunidade semelhante à população sem história prévia de infecção inoculada com duas doses. Saadat *et al* compararam os títulos de anticorpos IgG de profissionais de saúde com e sem infeção prévia por SARS-CoV-2.[2] Segundo os autores, os primeiros têm uma resposta secundária clássica à vacinação apenas com uma dose da vacina, com um pico de IgG em D14 pós-vacinação. No mesmo estudo, a neutralização vírica em laboratório permitiu afirmar com confiança que os profissionais com infeção COVID-19 prévia precisam apenas de uma dose da vacina. Estudos serológicos por Stamatatos *et al* demonstraram ainda que a imunidade após uma dose única em doentes previamente infetados é extensível à variante Beta (B.1.351) do SARS-CoV-2 da África do Sul.[3]

Krammer *et al* compararam valores de IgG de indivíduos com infeção prévia por SARS-CoV-2 com valores de IgG de indivíduos sem infeção prévia, conclui que a administração de apenas uma dose da vacina aos indivíduos com infecção prévia permite atingir uma imunidade 10 vezes maior do que a imunidade conseguida com duas doses da vacina em indivíduos sem infecção prévia.[4] Este aumento da reação imune reflete-se numa maior incidência de efeitos adversos da vacinação. No dia 12 de fevereiro a *Haute Autorité de Santé* Francesa emitiu uma recomendação que vai no sentido de vacinar apenas com uma dose da vacina os indivíduos que tenham tido infecção confirmada com COVID-19 há mais de três meses e de preferência seis meses após a infecção.[5]

Os valores de IgG 14-16 dias após a inoculação única com a vacina Moderna mostram que os nossos profissionais de saúde desenvolveram uma resposta imune semelhante aos indivíduos sem história prévia de infeção por COVID-19 que fizeram as duas doses da mesma vacina. Numa altura em que as vacinas são um bem escasso e que poderão sobrar doses em várias instituições, parece-nos importante alertar para esta alternativa. Ou seja, na impossibilidade de garantir que a dose sobrante terá o reforço protocolado passadas 3 - 4 semanas, deve ponderar-se a

Tabela 1 – Sintomas e valores de IgG dos profissionais de saúde

n	1	2	3	4	5	6	7	8
Categoria profissional	Médico	Enfermeiro	Enfermeiro	AAM	AAM	Enfermeiro	Enfermeiro	Enfermeiro
Tempo da infeção COVID-19 até vacina (Dias)	91	79	83	100	107	80	120	129
IgG* D0 (U)	0,3	3	2,7	2,3	4,5	2,6	2,4	3,1
IgG* D14-16 (U)	7,6	> 9	> 9	> 9	> 9	> 9	> 9	> 9
Sintomas da vacina	dor local	dor local cefaleia febre mialgias fadiga tonturas	dor local cefaleia febre mialgias fadiga tonturas	dor local cefaleia	dor local	dor local	dor local	dor local

* Euroimmun AG, ELISA SARS-CoV-2 IgG, Cat 2606, valor de *cut-off* de acordo com o fabricante (negativo: < 0,8; positivo: > 1,1)
AAM: auxiliares de ação médica

utilização dessas doses para a inoculação única de quem já teve infecção por COVID-19.

CONTRIBUTO DOS AUTORES

Todos os autores contribuíram de igual forma para a redação 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 actualizada 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 conflitos de interesses relacionados com o presente trabalho.

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Este trabalho não recebeu qualquer tipo de suporte financeiro de nenhuma entidade no domínio público ou privado.

REFERÊNCIAS

1. Covid19.min-saude.pt. Direção-Geral da Saúde. Norma nº 001/2021 de 14/01/2021 atualizada a 23/03/2021 [consultado 2021 abr 02]. Disponível em: https://covid19.min-saude.pt/wp-content/uploads/2021/03/Norma_001_2021_act_23_03_2021.pdf.
2. Saadat S, Tehrani ZR, Logue J, Newman M, Frieman MB, Harris AD, et al. Single dose vaccination in healthcare workers previously infected with SARS-CoV-2. JAMA. 2021;325:1467-9.
3. Stamatatos L, Czartoski J, Wan Y, Homad L, Rubin V, Glantz H, et al. A single mRNA immunization boosts cross-variant neutralizing antibodies elicited by SARS-CoV-2 infection. medRxiv 2021.01.30.2125084. doi: 10.1101/2021.02.05.21251182
4. Krammer F, Srivastava K, the PARIS team, Simon V. Robust spike antibody responses and increased reactogenicity in seropositive individuals after a single dose of SARS-CoV-2 mRNA vaccine. medRxiv 2021.01.29.21250653. doi: 10.1101/2021.01.29.21250653
5. Has-Sante.fr. Haute Autorité de Santé, France. Une seule dose de vaccin pour les personnes ayant déjà été infectées par le SARS-CoV-2. [consultado 2021 abr 02]. Disponível em: https://www.has-sante.fr/jcms/p_3237456/fr/une-seule-dose-de-vaccin-pour-les-personnes-ayant-deja-ete-infectees-par-le-sars-cov-2.

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The Stool Color Card as a Screening Tool for Biliary Atresia in the Digital Version of the Portuguese Child and Youth Health Booklet

O Cartão de Cores das Fezes como Instrumento de Rastreio da Atrésia Biliar na Versão Digital do Boletim de Saúde Infantil e Juvenil Português

Keywords: Biliary Atresia/diagnosis; Feces; Jaundice, Neonatal; Neonatal Screening
Palavras-chave: Atresia das Vias Biliares/diagnóstico; Fezes; Icterícia Neonatal; Rastreio Neonatal

Dear Editor

Biliary atresia (BA) is a rare entity (incidence in Europe of 1/18 000 live births) leading to great morbidity and mortality and represents the first indication for pediatric liver transplantation.[1] The long-term prognosis depends on the timing of bile flow restoration (Kasai portoenterostomy). One of the major determining factors of prognosis and survival of the native liver is surgical intervention before 45 days of life.[2]

Taiwan has one of the highest incidence rates of BA in the world. Therefore, a universal screening program was started there in 2002, using a validated stool color card (SCC). Subsequently, in 2011, it was observed that the median age of patients undergoing Kasai portoenterostomy decreased substantially, thus improving prognosis.[3] Meanwhile, other countries have demonstrated the cost-effectiveness of this tool,[4] and some have already started using it as a teaching and surveillance tool, by enrolling parents and caregivers. In Portugal, and as far as we know, there have been no initiatives at the level of the central healthcare system on this matter.

The SCC was originally created by Prof. Mei-Hwei Chang and her team, from the National University of Taiwan Medical College. It includes nine stool-colored images: three with normal colors and six with abnormal colors, which are considered red flags requiring urgent observation of the newborn/infant by Pediatricians.

A study performed in Northern Portugal has identified a significant percentage of healthcare professionals with clinical practices that may delay recognition of BA.[5] Recently, we have obtained written permission from both Prof. Mei-Hwei Chang and the Taiwan Ministry of Health and Health Promotion Administration to use the original SCC and its validated images for the purposes of a screening program in Portugal.

We propose the use of SCC as a teaching tool for parents at the time of discharge from the maternity hospital, and the inclusion of an early ambulatory screening in the first two visits after discharge by inserting the SCC into the Child and Youth Health Booklet. If the Portuguese Directorate-General of Health will convert the current paper booklet into a digital format, as it has been preliminarily admitted, we propose that a SCC should be part of this update (Fig. 1) and that a digital version should be accessible to both parents and caregivers, for example, through a web link or an app. In this scenario, the outcome of the implementation of this practice must be validated and a referral network should be implemented.

AUTHORS CONTRIBUTION

JA: Requested for permission to use the original SCC and its validated images, drafted the manuscript and approved its final version.

MT: Contributed with intellectual insights to the work, critically reviewed the manuscript and approved its final version.

ESS: Designed the work, supervised the request for

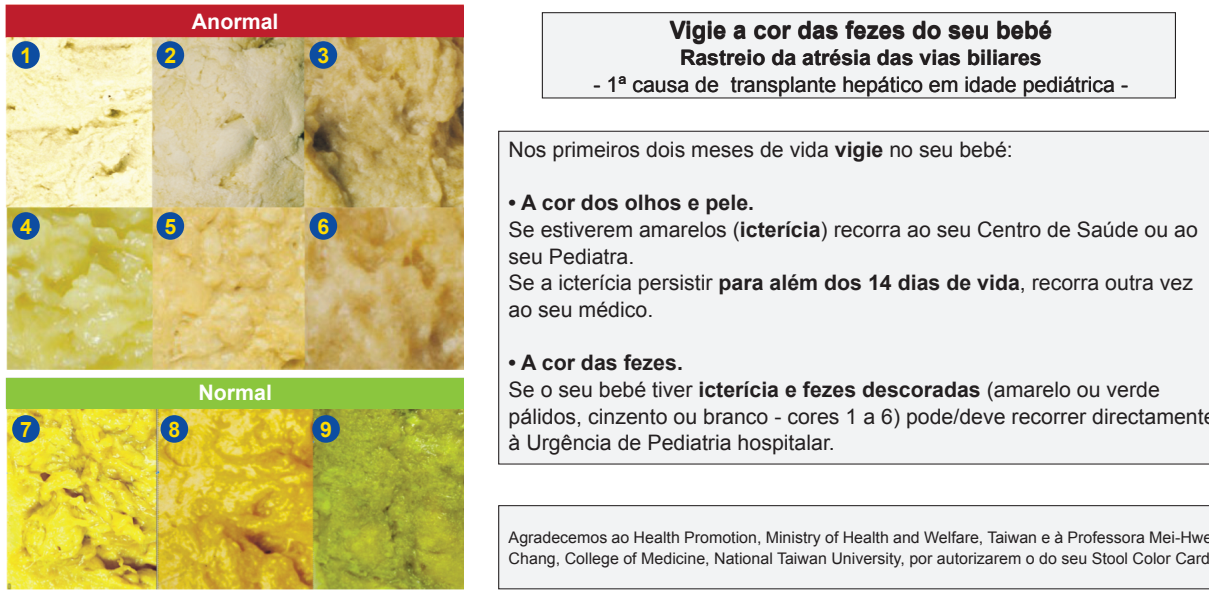

Figure 1 – Portuguese stool color card for screening biliary atresia in newborns and infants

permission to use the original SCC and its validated images, critically reviewed the manuscript and approved its final version.

AIL: Designed the work, critically reviewed the manuscript and approved its 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

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REFERENCES

1. Fanna M, Masson G, Capito C, Girard M, Guerin F, Hermeziu B, et al. Management of biliary atresia in France 1986 to 2015: long-term results. J Pediatr Gastroenterol Nutr. 2019;69:416-24.
2. Serinet MO, Wildhaber B, Broue P, Lachaux A, Sarles J, Jacquemin E, et al. Impact of age at Kasai operation on its results in late childhood and adolescence: a rational basis for biliary atresia screening. Pediatrics. 2009;123:1280-6.
3. Lien TH, Chang MH, Wu JF, Chen HL, Lee HC, Chen AC, et al. Effects of the infant stool color card screening program on 5-year outcome of biliary atresia in Taiwan. Hepatology. 2011;53:202-8.
4. Mogul D, Zhou M, Intihar P, Schwarz K, Frick K. Cost-effective analysis of screening for biliary atresia with the stool color card. J Pediatr Gastroenterol Nutr. 2015;60:91-8.
5. Santos Silva E, Moreira Silva H, Azevedo Lijnzaat L, Melo C, Costa E, Martins E, et al. Clinical practices among healthcare professionals concerning neonatal jaundice and pale stools. Eur J Pediatr. 2017;176:361-9.

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Letter to the Editor concerning "HIPTCN: Prospective Observational Study of Hypocoagulated Head Trauma Patients with Normal Admission Computed Tomography Scan"

Carta ao Editor referente a "HIPTCN: Estudo Prospetivo Observacional de Doentes Traumatizados Cranioencefálicos Hipocoagulados com Tomografia Computorizada Inicial Normal"

Keywords: Anticoagulants; Brain Injuries, Traumatic; Intracranial Hemorrhage, Traumatic; Neurosurgical Procedures; Tomography, X-Ray Computed
Palavras-chave: Anticoagulantes; Hemorragia Intracraniana Traumática; Lesões Encefálicas Traumáticas; Procedimentos Neurocirúrgicos; Tomografia Computorizada

Dear Editor,

Duarte-Batista *et al* have analyzed the occurrences and outcomes of a series of 178 adult patients with head injury that had an initial normal head computed tomography (CT) scan, and despite that, further remained in hospital for a 24-hour surveillance period due to a hypocoagulative state (drug induced and/or caused by coagulopathy), then undergoing a post surveillance CT scan. As a rule, anticoagulation medication was not taken during the surveillance period, they state, but the cases in which either partial or total reversal was performed were not reported. With this approach, the authors found that the mortality rate of patients was zero.[1] They diagnosed all the patients that developed delayed intracranial hemorrhage (n = 4, 2.3%), and curiously they were all asymptomatic; one had already undergone reversal of anticoagulation and the remaining were kept off the medication. None of these patients with delayed intracranial hemorrhage required surgery, and no morbidity was recorded. They also state that their results cannot support exclusion of post surveillance CT scan since it dictated the management of anticoagulant therapy. They also recall the possibility of a falsely normal initial head CT scan that has been described in the literature in this setting due to errors in radiology reporting.

Nevertheless, they assume seven occurrences that they encountered during the 24 hour in-hospital surveillance period as complications caused by this in-hospital requirement, and in view of this 3.9% rate, they hypothesize on home surveillance and ambulatory post surveillance CT scan. They report five situations of mental changes (agitation, confusion) as maladaptation of the patient to the hospital environment, but a differential diagnosis with brain concussion symptoms does not seem to have been made. A case of stridor due to nasogastric intubation attempt was described, but the indication for the procedure was apparently judged as correct; in a home setting this could lead to more serious consequences. The case of atrial fibrillation with tachycardia due to withdrawal of beta blockers regarding which the medical team was unaware of could happen in the home setting as well, especially when dealing with a population with a mean age of 80.7 years as reported by the authors. The proposal of home surveillance and proper information for patients and caregivers regarding red flags in selected situations does not provide clues as to how effectively can the healthcare team in an emergency room setting ascertain the real aptitude of both patients and caregivers to perform these tasks. The patients might be subjected to transportation constraints in the setting of acute trauma, and the organization of prehospital emergency care can feel like an extra burden if the patients deteriorate at home and have to be transported back to the hospital, either for due and undue reasons. The authors also do not elaborate on the importance and merit of diagnosing a worsening Glasgow coma scale score in four patients (2.2%) and new neurological deficits in two (1.1%) during the in-hospital surveillance period, and on the possible consequences of this occurring in a home setting.

A seminal paper on this subject[2] reports on four patients who had normal neurological examinations and normal computed tomographic scans after mild head injury, and who later developed an acute subdural hematoma and deteriorated rapidly. One deteriorated 12 hours after the trauma, two on the day after, and the other on day three. Three of the four patients underwent craniotomy for evacuation of their hematomas. Two patients died and two reached Glasgow outcome scores of three and four only after extended inpatient rehabilitation. None of these patients underwent reversal / correction of the coagulation abnormalities, one was discharged right after trauma, another six hours after, and the remaining two were inpatients. The repeat CT scan was performed due to the neurological deterioration. This serves to illustrate that such favorable outcomes as reported by Duarte-Batista *et al* are not necessarily the rule for these patients; that consideration should be given to a full evaluation of the coagulation status of these patients; that suspension of these medicines and even early reversal in situations of largely abnormal laboratory results should be considered; and that a repeat CT scan within a six to 24 hour time frame could allow for the timely diagnosis of delayed intracranial traumatic hemorrhage in hypocoagulated patients.

Duarte-Batista *et al* are to be commended since they have succeeded in the Herculean task of performing a multicenter study on hypocoagulated head trauma patients with normal admission CT scan, and demonstrating that very low morbidity and mortality rates can be achieved in the management of these patients with the protocol that they use. Changes to that protocol can certainly be hypothesized, but further variables should be brought into the discussion.

COMPETING INTERESTS

The author states there are no competing interests.

FUNDING SOURCES

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REFERENCES

1. Duarte-Batista P, Farinha N, Marques R, Pinheiro J, Silva J, Tuna, R, et al. HIPTCN: prospective observational study of hypocoagulated head trauma patients with normal admission computed tomography scan. Acta Med Port. 2021;34:413-9.
2. Itshayek E, Rosenthal G, Fraifeld S, Perez-Sanchez X, Cohen JE, Spektor S. Delayed posttraumatic acute subdural hematoma in elderly patients on anticoagulation. Neurosurgery. 2006;58:E851-6.

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Outpatient Community-Acquired Pneumonia: Out of Sight, Away from Prevention

Pneumonia Adquirida na Comunidade em Ambulatório: Longe da Vista, Longe da Prevenção

Keywords: Ambulatory Care; Community-Acquired Infections; Pneumonia
Palavras-chave: Cuidados em Ambulatório; Infecções Adquiridas na Comunidade; Pneumonia

Dear editor,

Community-acquired pneumonia (CAP) is a common and potentially serious illness with a significant morbidity and mortality rate. The estimated annual incidence of CAP is five to 11 cases per 1000 adults and incidence increases with age and seems to be higher in men than in women.[1] CAP remains one of the most common medical causes of hospital admission in healthcare systems[2] and in Portugal, between 2000 - 2009, it was responsible for 3.7% of the hospitalizations.

After diagnosis, the initial management decision is to determine the location of care: outpatient, hospitalization, or admission to an Intensive Care Unit (ICU). It is essential to avoid unnecessary admissions to avoid the costs and higher consumption of resources and minimize the risk of nosocomial infections by resistant hospital bacteria or thromboembolic events.[3]

The aim of this study was to determine the incidence of CAP in a Portuguese adult outpatient population (over 18 years old) and to characterize those patients, their risk factors and respective vaccination status (seasonal flu and pneumococcal vaccination).

This was a prospective, multi-center and observational study carried out in the ambulatory setting. It involved pulmonologists from Centro Hospitalar Universitário Lisboa Norte with the collaboration of USF-AN (Associação Nacional das Unidades de Saúde Familiar) and family physicians from three Health Units in Northern Portugal. The study was approved by the respective Regional Health Administrations and Ethics Committees. A total of 27 711 adults were observed by their family doctor, over a period of one year (between the 1st July 2014 to the 30tth June 2015). We prospectively analyzed all the diagnoses of CAP that were coded with the ICPC-2® code R81 in the problem list and which corresponded to a diagnosis of CAP (viral and bacterial pneumonia, bronchopneumonia and legionnaires' disease, excluding aspiration pneumonia and obstructive pneumonia associated with lung cancer). The inclusion criteria were age over 18 years old and a diagnosis of CAP made according to the standard of care based on clinical and radiological features. The exclusion criteria were patients aged under 18 years old and patients with HIV infection.

In this study, 33 patients with a CAP diagnosis were included, corresponding to an incidence of 1.19 per 1000 adults. The average age was 65.4 years, ranging from 20 to 96 years of age and there were 18 women (54.5%).

Fifteen (45.5%) patients went directly to the hospital emergency room (ER). The family physician was later informed of the diagnosis. In the remaining 19 (54.5%) patients, the diagnosis was made in a primary health care facility. Four of these patients required hospital referral and no patients required hospitalization or died.

In terms of smoking and alcohol habits, 21 (63.6%) never smoked, 12 (36.4%) had past or active smoking habits, eight (24.2%) consumed alcohol regularly and 25 (75.8%) did not usually drink alcohol. As for vaccination, nine (27.3%) received seasonal flu vaccination (all over 65 years old and entitled to free flu vaccination), while 23 (70%) did not receive any vaccination. There was only one patient (3.0%) that received inoculation with pneumococcal polysaccharide vaccine (PPSV23) and the other 32 (96.7%) did not receive any inoculation of the PPSV23 vaccine. None of the 33 cases received the pneumococcal 13-valent conjugate vaccine. Sixteen patients (48.5%) had, at least, one comorbidity (Table 1).

The main limitation of this study lies in the retrospective diagnosis in almost half of the patients along with the possibility that other cases of CAP may not have been reported. This may compromise some results, namely the low number of cases, low prevalence of smoking and alcohol drinking habits and predominance of female patients. The latter can be explained by the fact that, in general, women consult their family physician more often.

The low vaccination coverage for both the influenza andpneumococcal vaccines should be mentioned. The population that was vaccinated against influenza was included in the groups entitled to free vaccination and without the need for medical consultation, which may explain the difference compared to the pneumococcal vaccine.

According to the expected annual incidence of five to 11 cases per 1000 adults, between 138 and 305 cases of CAP should have been diagnosed in this study, almost four to nine times more than the 33 reported cases. This low number of outpatient diagnoses, compared to the number of hospital admissions, suggests that most patients with

Table 1 – Comorbidities in patients with a diagnosis of CAP (some patients may have multiple comorbidities)

Comorbidities	n (%)
Any comorbidity	16 (48.5%)
Obesity (grades 1, 2, 3)	11 (33.3%)
Renal failure	7 (21.2%)
Diabetes mellitus	6 (18.2%)
Cardiac failure	5 (15.2%)
Cancer	4 (12.1%)
Asthma	3 (9.1%)
COPD	3 (9.1%)
Hepatic disease	1 (3.0%)

CAP sought care directly in the ER, instead of seeking their family physician first.

This diversion of patients from Primary Health Care directly to the hospital ER with reduced monitoring of patients with pneumonia by family physicians can make it more difficult to implement preventive measures, namely flu and pneumococcal vaccination.

The impact and severity of CAP justify additional studies in the outpatient setting that would allow a better characterization of this disease and promote a greater awareness for its prevention.

AUTHORS CONTRIBUTION

All authors contributed equally to the concept, draft, review and edition of the work.

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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REFERENCES

1. Welte T, Torres A, Nathwani D. Clinical and economic burden of community-acquired pneumonia among adults in Europe. Thorax. 2012;67:71–9.
2. Wunderink RG, Waterer G. Advances in the causes and management of community acquired pneumonia in Adults. BMJ. 2017;358:2471.
3. Mandell L, Wunderink R, Anzueto A, Bartlett J, Douglas Campbell G, Dean N, et al. Infectious Diseases Society of America/American Thoracic Society Consensus Guidelines on the Management of Community-Acquired Pneumonia in Adults. IDSA/ATS Guidelines for CAP in Adults; CID 2007:44.

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Reverse Transcription Polymerase Chain Reaction Pattern of SARS-CoV-2 Beta Variant

Padrão da Variante Beta do SARS-CoV-2 por Biologia Molecular (RT-PCR)

Keywords: COVID-19/epidemiology; COVID-19/virology; Mutation; Portugal; Reverse Transcriptase Polymerase Chain Reaction; SARS-CoV-2
Palavras-chave: COVID-19/epidemiologia; COVID-19/virologia; Mutação; Portugal; Reação em Cadeia da Polimerase Via Transcriptase Reversa; SARS-CoV-2

To the Editor:

In March 2021, the presence of the SARS-CoV-2 Beta (B.1.351) variant was suspected and detected in our hospital by real-time reverse transcription polymerase chain reaction (RT-PCR), later confirmed by the National Institute of Health Dr. Ricardo Jorge (INSA) by genomic sequencing. With the increase in the prevalence of SARS-CoV-2 variants in Portugal[1] and the fact that not all laboratories have specific kits to detect these mutations, we believe that it is relevant to share the findings that led us to suspect the presence of a variant in these patients. This variant is considerably more contagious and can quickly worsen the underlying disease of inpatients that share the same ward if they become infected.[2]

The RT-PCR test can be performed using various kits, which can detect various viral genes. These genes code for different structures such as the spike glycoprotein (S), the envelope protein (E), the nucleoprotein (N) and the ORF1ab sequence that contains RNA-dependent RNA polymerase (RdRP).[3]

Among the SARS-CoV-2 variants currently in circulation in Portugal, there are some raising attention. The Alpha (B.1.1.7) variant, originally identified in the United Kingdom in September 2020, the Gamma (P.1) variant circulating in Brazil since mid-2020 and the Beta (B.1.351) variant described in South Africa at the end of 2020.[4] The three variants share the *N501Y* mutation and the Beta and Gamma variants share the *E484K* mutation.[5]

Samples from our patients, obtained by nasopharyngeal swabs, were tested using the Allplex™ (Seegene®; Werfen) RT-PCR kit that detects the presence of the E, RdRP/S and N genes. The samples had low cycle thresholds (Ct) values (< 25 - 30) and we noticed a RT-PCR amplification pattern in which the N gene amplified two to six cycles later in relation to the other genes. This raised the suspicion that these samples could contain a SARS-CoV-2 variant. The results are described in Table 1.

In order to clarify these findings, we tested the samples with the Novaplex™ I (Seegene®, Werfen) RT-PCR kit that can detect the presence of the specific mutations in each variant. All samples were positive for the *E484K* and *N501Y* mutations and negative for the 69/70 deletion. In order to differentiate the Gamma and Beta variants, samples were sent to *INSA* for genome sequencing, which revealed that all samples had the SARS-CoV-2 Beta variant (B.1.351) Therefore, we identified 12 new cases of the Beta mutation. Until March 2021, there were only five reported cases of this variant in Portugal. In the most recent report, there are 103 reported cases.[1]

The aim of this letter is to raise awareness regarding this variant, which can be suspected when there are lower cycle threshold values and the N gene has two to six amplification cycles later than the other genes tested, which is relevant since genome sequencing is a time consuming process. Kits that detect SARS-CoV-2 variants should be implemented as reflex testing when this pattern is present.

AUTHORS CONTRIBUTION

DFS: Draft of the paper, critical review, and approval of the final version of the paper.

PDS: Draft of the paper and critical review.

IB: Critical review and approval of the final version of the paper.

Table 1 – Real-Time RT-PCR results

Kit (Analyzer)	**Samples**	**Gene**		
		E (ct)	**RdRP/S** (ct)	**N (ct)**
AllplexTM (Seegene®, Werfen)	A	15.17	17.36	22.53
	B	20.16	22.08	25.89
	C	18.29	18.20	21.29
	D	16.06	15.77	20.87
	E	19.06	18.03	22.87
	F	19.57	18.98	22.23
	G	20.85	20.93	27.14
	H	22.17	22.59	26.43
	I	19.80	20.05	25.90
	J	20.35	20.70	25.00
	K	26.21	25.74	31.49
	L	23.03	21.75	25.20

ct: cycle threshold

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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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REFERENCES

1. Instituto Nacional de Saúde Doutor Ricardo Jorge. Diversidade genética do novo coronavírus SARS-CoV-2 (COVID-19) em Portugal, 31 de maio de 2021. Lisboa: INSA; 2021.
2. Janik E, Niemcewicz M, Podogrocki M, Majsterek I, Bijak M. The emerging concern and interest SARS-CoV-2 variants. Pathogens. 2021;10:633.
3. Naqvi A, Fatima K, Mohammad T, Fatima U, Singh I, Singh A, et al. Insights into SARS-CoV-2 genome, structure, evolution, pathogenesis and therapies: structural genomics approach. Biochim Biophys Acta Mol Basis Dis. 2020;1866:165878.
4. Burki T. Understanding variants of SARS-CoV-2. Lancet. 2021;397:462.
5. Wu K, Werner AP, Moliva JI, Koch M, Choi A, Stewart-Jones GB, et al. mRNA-1273 vaccine induces neutralizing antibodies against spike mutants from global SARS-CoV-2 variants. bioRxiv. 2021:2021.01.25.427948.

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Gastrointestinal Symptoms and Liver Injury on Admission in a Hospitalised Population with COVID-19 Infection

Sintomas Gastrointestinais e Lesão Hepática à Admissão numa População Hospitalizada com Infecção por COVID-19

Keywords: COVID-19; Gastrointestinal Diseases; Liver Diseases; SARS-CoV-2
Palavras-chave: COVID-19; Doenças do Fígado; Doenças Gastrointestinais; SARS-CoV-2

Dear Editor,

The novel coronavirus disease 2019 (COVID-19) may present with gastrointestinal symptoms and liver injury, but current evidence on their prevalence and association with disease-severity and severe outcomes is conflicting.[1–3]

We retrospectively analysed the prevalence of gastrointestinal manifestations and elevated liver enzymes in adult patients hospitalised in wards of a tertiary hospital in Portugal, with a COVID-19 diagnosis between March and August 2020. We studied their association with hospital mortality, intensive care unit (ICU) admission and hospital length of stay. The study was approved by the hospital's Ethics Committee.

A total of 561 patients were selected, mean aged 62 ± 20 years and 63% men. After excluding 25 patients due to previous gastrointestinal or liver disease, 536 patients were eligible for analysis. Gastrointestinal symptoms were present in 22% and included diarrhoea (14%), nausea/vomiting (10%) and abdominal pain/discomfort (5%). Elevated liver enzymes on admission were found in 30% of the patients. Seventeen percent (n = 93) presented with an isolated aspartate transaminase (AST) elevation [median 49 (43;62) UI/L] and 13% (n = 70) with both AST and alanine aminotransferase (ALT) elevated [median 66 (51;10) UI/L and 83 (65;109) UI/L, respectively], with a mean AST/ALT ratio of 0.94 ± 0.48.

We found an association between liver enzyme changes and mortality in multivariable analysis adjusted for age, gender and patients' comorbidities such as hypertension, obesity, diabetes mellitus and respiratory disease. Patients with isolated AST elevation and with both AST and ALT elevations were associated with greater hospital mortality [odds ratios (OR) of 2.28 (95% CI 1.19 - 4.35, p = 0.012) and 2.68 (95% CI 1.12 - 6.45, p = 0.028), respectively], compared to patients without liver changes. The presence of gastrointestinal symptoms had no statistically significant association with hospital mortality (p = 0.078) or ICU admission (p = 0.750). Patients with isolated AST elevation had an OR of 2.33 (95% CI 1.37 - 3.98, p = 0.002) of being admitted to an ICU than patients without liver changes, but these differences were not seen for patients with elevation of both enzymes (OR 1.65, 95% CI 0.88 - 3.09, p = 0.119). Median hospital length of stay was 10 (5; 12) days, without differences between groups with gastrointestinal symptoms (p = 0.305) or elevated liver enzymes (p = 0.259).

In our Portuguese cohort, the prevalence of gastrointestinal symptoms and liver injury on presentation was similar to that of previous reports. Several mechanisms for these changes have been proposed, including the direct cytopathic effect of the virus mediated by ACE-2 receptors, which are highly expressed in intestinal epithelial cells, hepatocytes and cholangiocytes, and injury mediated by ischaemia and systemic inflammatory response in patients presenting with severe infection.[4,5]

While gastrointestinal manifestations were unrelated to worse outcomes, liver injury on admission, characterized by moderate AST and ALT elevations, was associated with greater hospital mortality. This should raise awareness to the possible prognostic value of elevated liver enzymes on admission in patients infected with SARS-CoV-2.

AUTHORS CONTRIBUTION

ROS, MIC: Data acquisition, statistics analysis, draft of the manuscript.

VA, JF, AD: Data acquisition.

ACR, DC, FM, AP, JC: Critical review of the paper.

DATA CONFIDENTIALITY

The authors declare having followed the confidentiality rules of their working centre regarding patients' data collection. Data was collected and stored in a pseudonymized database for statistical analysis. The authors further declare this work followed the regulations established by the Helsinki Declaration of the World Medical Association updated in 2013.

COMPETING INTERESTS

The authors declare no conflict of interest.

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REFERENCES

1. Parasa S, Desai M, Thoguluva Chandrasekar V, Patel HK, Kennedy KF, Roesch T, et al. Prevalence of gastrointestinal symptoms and fecal viral shedding in patients with coronavirus disease 2019: a systematic review and meta-analysis. JAMA Netw open. 2020;3:e2011335.
2. Wang H, Qiu P, Liu J, Wang F, Zhao Q. The liver injury and gastrointestinal symptoms in patients with coronavirus disease 19: A systematic review and meta-analysis. Clin Res Hepatol Gastroenterol. 2020;44:653–61.
3. Cai Q, Huang D, Yu H, Zhu Z, Xia Z, Su Y, et al. COVID-19: abnormal liver function tests. J Hepatol. 2020;73:566–74.
4. Zhong P, Xu J, Yang D, Shen Y, Wang L, Feng Y, et al. COVID-19-associated gastrointestinal and liver injury: clinical features and potential mechanisms. Signal Transduct Target Ther. 2020;5:256.
5. Schaefer EA, Arvind A, Bloom PP, Chung RT. Interrelationship between coronavirus infection and liver disease. Clin Liver Dis. 2020;15:175–80.

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New-Onset Type 1 Diabetes in Children and COVID-19

Diabetes Tipo 1 Inaugural em Idade Pediátrica e COVID-19

Keywords: Child; COVID-19; Diabetes Mellitus, Type 1; SARS-CoV-2
Palavras-chave: COVID-19; Criança; Diabetes Mellitus Tipo 1; SARS-CoV-2

Dear Editor,

During the COVID-19 pandemic, a lower rate of healthcare care usage has been reported, like for example admissions to Pediatric Emergency Departments. This can lead to the delayed diagnosis of potentially severe diseases, like type 1 diabetes mellitus (DM). Previous studies from Italy and Germany found an increase in severe diabetic ketoacidosis (DKA) at the time of DM diagnosis in children during the COVID-19 pandemic.[1,2]

In our observational and retrospective study, we evaluated the frequency and severity of new-onset DM in children, at a tertiary hospital in Lisbon, during the first year of the COVID-19 pandemic (April 2020 – March 2021) and compared them with a similar period, prior to the COVID-19 outbreak in Portugal (April 2019 – March 2020). The independent *t*-test, Mann-Whitney test and the chi-square test were used, where appropriate. Statistical significance was predetermined as $p < 0.05$. SPSS® 26 was used for statistical analysis. Ethical approval was not required by the Ethics Committee of Centro Hospitalar Lisboa Norte-Hospital de Santa Maria (Lisbon, Portugal) for the present study, since it was based on retrospective data collection. The results are shown in Table 1.

Between April 2020 and March 2021, 20 children were diagnosed with type 1 DM, a lower number than in previous years. The median age was similar between the two groups, as well as glycemia and symptoms at presentation. Only one child presented simultaneously with SARS-CoV-2 infection.

A significantly higher proportion of children presented with severe DKA during the COVID-19 pandemic. Arterial blood gas parameters such as pH, bicarbonate, and base excess were also worse in this group of patients. Consequently, more children were admitted to the intensive care unit. Nonetheless, the reported median duration of preceding symptoms of DM was not statistically different between the two groups and was, in fact, shorter in the COVID-19 pandemic group.

In conclusion, during the first year of the pandemic, we observed a significant increase in severe cases of DKA. The notion of delay in healthcare seeking to explain this increase could not be established. However, the duration of symptoms was self-reported, which may limit the conclusions. It is also likely that complex psychosocial factors related with social isolation could have changed the perception of symptoms of the disease.[3] Recent studies suggest that SARS-CoV-2 can act as an infectious trigger and precipitate DKA in patients with new-onset DM.[4,5] However, in our cohort, only one patient had SARS-CoV-2 infection and patients had not been tested for previous exposure through serological tests.

Further research into the causes of the increase in DKA during the pandemic is required. Additionally, strategies to educate parents about timely attendance at the emergency department remain crucial.

AUTHORS CONTRIBUTION

MIA: Data aquisition, draft of the paper.
ARH, LR: Data aquisition, critical review of the paper.

Table 1 – Comparison of clinical and biochemical parameters between the COVID-19 group and the non-COVID-19 group

		April 2020 - March 2021 (COVID-19 pandemic)	April 2019 - March 2020	*p* value
	Number of patients	20	27	-
	Age, years	10.0 (7.0)	12.3 (5.2)	0.220
	Sex	45% male	63% male	0.221
Reported symptoms	Days of preceding symptoms	18.0 (88.0)	30.0 (147.0)	0.266
	Polydipsia	90%	96%	0.383
	Polyuria	85%	100%	0.038
	Polyphagia	35%	56%	0.163
	Weight loss	90%	82%	0.417
	Mean % of weight loss	-10.5% ± 8.85	-10.0% ± 6.42	0.826
	ICU stay	30%	3.7%	**0.012**
Blood tests at admission	Glycemia, mg/dL	458 (195.0)	493 (225.0)	0.426
	pH	7.1 (0.315)	7.31 (0.176)	**0.007**
	Bicarbonate, mmol/L	9.4 (11.0)	18.45 (9.6)	**0.03**
	Excess base, mmol/L	-21.1 (18.2)	-7.2 (14.6)	**0.01**

ICU: intensive care unit
Values are given as n or %, mean ± standard deviation or median (interquartile range)

DC, SC: Data aquisition, statistics analysis.
BR, CP, MLS: Critical review of 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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REFERENCES

1. Kamrath C, Mönkemöller K, Biester T, Rohrer TR, Warncke K, Hammersen J, et al. Ketoacidosis in children and adolescents with newly diagnosed type 1 diabetes during the COVID-19 pandemic in Germany. JAMA. 2020;324:801-4.
2. Rabbone I, Schiaffini R, Cherubini V, Maffeis C, Scaramuzza A; Diabetes Study Group of the Italian Society for Pediatric Endocrinology and Diabetes. Has COVID-19 delayed the diagnosis and worsened the presentation of type 1 diabetes in children? Diabetes Care. 2020;43:2870-2.
3. Tittel SR, Rosenbauer J, Kamrath C, Ziegler J, Reschke F, Hammersen J, et al. DPV Initiative. Did the COVID-19 lockdown affect the incidence of pediatric type 1 diabetes in Germany? Diabetes Care. 2020;43:e172-3.
4. Boddu SK, Aurangabadkar G, Kuchay MS. New onset diabetes, type 1 diabetes and COVID-19. Diabetes Metab Syndr. 2020;14:2211-7.
5. Unsworth R, Wallace S, Oliver NS, Yeung S, Kshirsagar A, Naidu H, et al. New-onset type 1 diabetes in children during COVID-19: multicenter regional findings in the U.K. Diabetes Care. 2020;43:e170-1.

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Prevalence of Pediatric Vestibular Disorders

Prevalência da Doença Vestibular Pediátrica

Keywords: Child; Vestibular Diseases/epidemiology
Palavras-chave: Criança; Doenças Vestibulares/epidemiologia

It was with great interest that we read the article by Lima AF *et al*.[1] The authors investigated vertigo complaints in children under 18 years of age that were referred to a tertiary academic center.

The reported prevalence and etiologies of vestibular disorders can vary depending on the hospital department or clinic to which children are referred to, the referral criteria, the age range of the patients tested and the vestibular testing technologies that are available.

We have carried out a specific pediatric dizziness clinic in a tertiary academic center with a specialized pediatric hospital with most cases referred from Ears, Nose and Throat (ENT) consultants. From January 2014 to December 2019, we attended to 78 new pediatric patients with dizziness complaints. Our diagnoses differ substantially from the diagnoses in the study by Lima *et al*, especially in terms of the number of cases of psychogenic vertigo and benign paroxysmal positional vertigo (BPPV) (Table 1). Psychogenic vertigo is a prevalent diagnosis, especially in adolescent girls. As for primary BPPV, it is a very rare condition in children and anterior canal BPPV is even more so.[2,3] We suspect that the cause for these differences might be a selection bias caused by nonconsecutive patient recruitment, as well as the difficulty in obtaining an accurate diagnosis in younger children.

We would also like to highlight the importance of the ophthalmic exam in pediatric patients, as this is the second most frequent cause of dizziness complaints in big centers and an aggravating factor in patients with the typical migraine equivalents.[4]

As for videonystagmography, we would like to stress that it is not the same as to performing caloric tests, and that in children, the rotatory chair is a very important test, especially in the early years of age.[4,5]

Regarding the patients diagnosed with vestibular neuritis, we do not understand why they all underwent imaging. If the head impulse, nystagmus, and Test-of-Skew - HINTS Protocol is followed as stated, the clinical history and physical examination should be the key elements to select which children with vertigo require neuroimaging.[4] When caloric testing was carried out later and showed no hypofunction, it makes us wonder if these were rather cases of first attacks of vestibular migraine, even though we acknowledge that follow-up at one year shows a very high recovery rate.[5] The authors did not mention the follow-up time.

AUTHORS CONTRIBUTION

AMA: Draft of the paper.

JCR: Critical review and approval of the final version of 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

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Table 1 – Pediatric population observed during a 5-year period in the Vestibular Clinic of the Hospital and University Centre of Coimbra - Pediatric Hospital

	Total (n = 78)
Sex (female, n%)	45 (57.7)
Age (mean, min - max)	12.3 (14 - 17)
Diagnosis (n, %)	
Vestibular migraine	23 (29.5)
Psycogenic	13 (16.5)
Vestibular neuritis	13 (16.7)
BPVC	3 (3.8)
BPPV	2 (2.6)
Genetic syndrome	5 (5.1)
Mix diagnosis	11 (14.1)
Unknown peripheral vestibular disease	9 (12.8)

BPVI: benign paroxysmal vertigo of childhood; BPPV: benign paroxysmal positional vertigo

REFERENCES

1. Lima A, Moreira F, Menezes A, Costa I, Azevedo C, Vilarinho S, et al. Patologia vestibular em idade pediátrica: análise retrospetiva e revisão da literatura. Acta Med Port. 2021;34:428-34.
2. Lee J, Kim C, Hong S, Kim S, Suh M, Kim M, et al. Prevalence of vestibular and balance disorders in children and adolescents according to age: a multi-center study. Int J Pediatr Otorhinolaryngol. 2017;94:36-9.
3. Wiener-Vacher S, Quarez J, Priol A. epidemiology of vestibular impairments in a pediatric population. Semin Hear. 2018;39:229-42.
4. Kattah J. Use of HINTS in the acute vestibular syndrome. An overview. Stroke Vasc Neurol. 2018;3:190-6.
5. Wiener-Vacher S. Troubles de l'équilibre et vertiges de l'enfant. EMC - Otorhinolaryngol. 2016;11:1-10.

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