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Original Article

Lack of correlation between surface water area and infection with *Pseudomonas aeruginosa* and the non-tuberculous mycobacteria (NTMs) in patients with cystic fibrosis (CF)

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A R T I C L E I N F O

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A B S T R A C T

Background: People with cystic fibrosis (CF) may develop clinically significant chronic respiratory infections with *Pseudomonas aeruginosa* (PA) and non-tuberculous mycobacteria (NTM). Open water has been suggested to be an important source for continuous or intermittent exposure to these pathogens. To date, there has been a paucity of studies examining the relationship between chronic PA and NTM infection in CF patients and surfaces waters, including blue spaces. The aim of this study was therefore to examine the relationship between chronic pulmonary infection with PA and NTMs in children and adults with CF in European countries and area of surface waters, including blue spaces.

Methods: European CF registry data detailing incidence of chronic PA and NTM infection in adults and children with CF in Europe (n=41,486 in 24 European countries) was correlated with surface water area data from the same countries (approx. 678,278 km^2) employing Spearman coefficients.

Results: Correlation of chronic PA infection in children and adults and surface water area were not significant (p=0.0680 and p=0.8448, respectively), as was NTM infection (p=0.7371 and p=0.0712, respectively).

Conclusions: Acquistion of PA and its avoidance in people with CF is a complicated dynamic, not solely driven by close association with surface water, but through the integration of several other factors, including mitigations by people with CF to avoid high risk scenarios with surface water. This study was unable to demonstrate a correlation between PA and NTM infection in people with cystic fibrosis and surface water area at a national level. CF patients should continue to be vigilant about potential infection risks posed by water and take evidence-based decisions regarding their behaviour around water to protect them for acquiring these organisms from these sources.

1. Introduction

Cystic fibrosis (CF) is an inherited human genetic disorder caused by mutations in the gene that codes for the cystic fibrosis transmembrane conductance regulator (CFTR) protein, which results in the malfunction of the CFTR protein [1]. The dysfunction of the CFTR protein leads to the development and accumulation of thick mucus, particularly in the lungs, which greatly restricts mucociliary clearance. For a full description on the pathophysiology of CF, please see the review by Shteinberg and colleagues [1]. Such limited or absence of CFTR functionality contributes to creating conditions for commensal, environmental and pathogenic bacterial, particularly including *Pseudomonas aeruginosa* (PA) and the non-tuberculous mycobacteria (NTMs), to colonise, survive and persist, potentially leading to chronic infection [2]. As a result, people with CF are particularly vulnerable to lung infections,

Abbreviations: CF, cystic fibrosis; CFTR, cystic fibrosis transmembrane conductance regulator; NTM, non-tuberculous mycobacteria; PA, *Pseudomonas aeruginosa*.

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which are responsible for increased morbidity and mortality, where pulmonary infection continues to be the main cause of death [2].

Several studies and reports describe the sources and reservoirs of PA and NTMs for CF patients, in an attempt to educate patients on where these organisms are found in the environment and how to modify behaviours to minimise the risk of exposure to these sources, in an attempt to avoid pathogen acquistion [3,4,5]. A network visualisation map shows the linkages in publications between the search terms "water" and "cystic fibrosis" employing VOSviewer (version 1.6.19) (https://www.vosviewer.com) [6] (Supplementary File 1). Waters, including shower, drinking and surface water have been reported as important environmental sources of these organisms [7,8]. Open water has been suggested to be an important reservoir and source for continuous or intermittent exposure to PA [9].

More recently, the term "*blue spaces*" has been employed, where these are defined as "*outdoor environments–either natural or manmade–that prominently feature water and are accessible to people*" [10]. Overall, "*blue space*" is the collective term for rivers, lakes, canals, or the sea, however the present study was solely interested in the surface area of fresh water and not the marine environment.

To date, there has been a paucity of studies examining the relationship between chronic PA and NTM infection in patients with CF and the area of surface water in the European country that they live in. Increased occurrence of surface freshwater area may increase the risk of contact with pathogens in the water through water-related activities. Water sports include those activities which take place, (i) on the water (n=32), (ii) in the water (n=13) and (iii) under the water (n=20) [11], where popular water sports include water skiing, cold water swimming, white water rafting, bouldering, deep water soloing, kayaking, canoeing, sailing, windsurfing, angling. In the UK, recent studies have shown that watersports participation is rising, where a survey conducted by a consortium of leading marine bodies including British Marine, Royal Yachting Association (RYA), Maritime and Coastguard Agency (MCA), Royal National Lifeboat Institution (RNLI), British Canoeing (BC) and the Centre for Environment, Fisheries and Aquaculture Science (Cefas), indicated that approximately 3.9 m UK adults took part in one or more boating activities in 2018 [12].

Acquistion of organisms may be through aerosolisation of pathogens from waterways, especially rivers and increased recreational involvement with rivers, including walking, cycling and running along waterways and watercourses, especially if the natural waterway is a source of aerosolisation, e.g. rapids and particularly waterfalls. Therefore, the hypothesis of this study was that increased exposure of people with CF to surface water could result in infection with PA and NTMs and infection rates would be proportional to surface area of water. The aim of this study was therefore to examine the relationship between chronic pulmonary infection with PA and NTMs in children and adults with CF in 24 European countries and area of surface waters from respective countries.

2. Methods

Data relating to the combined area of surface waters (km^2), including all water bodies (rivers, canals, lakes) in 24 European countries was collected from the WISE-Freshwater gateway database, EU Commission/European Environment Agency (WISE- Freshwater. Freshwater Information System for Europe. https://water.europa.eu/freshwater/data-maps-and-tools/water-framework-directive-surface-water-data-products/delineation-surface-water). WISE-Freshwater is a gateway for searching, accessing, retrieving and understanding data and information on the environmental status and policy assessments of European freshwaters (surface- and groundwaters). Total area of surface water was recorded in km^2 for each of the 24 countries examined, including Austria, Belgium, Bulgaria, Croatia, Cyprus, Czech Republic, Denmark, France, Germany, Greece, Hungary, Ireland, Italy, Latvia, Lithuania, Netherlands, Norway, Poland, Portugal, Romania, Slovenia, Spain, Sweden and UK.

Prevalence rates of chronic *Pseudomonas aeruginosa* and non-tuberculous mycobacteria infection in people with cystic fibrosis, for each of the 24 European countries listed above was obtained from the European Cystic Fibrosis Society Patient Registry: 2019 Annual Data Report (https://www.ecfs.eu/sites/default/files/general-content-files/working-groups/ecfs-patient-registry/ECFSPR_Report_2019_v1_16Feb2022.pdf). Data was obtained separately for children and adults. 2019 data was employed to avoid potential artefactual anomalies introduced into reporting during the COVID-19 pandemic years (2020-2022).

2.1. Statistical analyses

Data underwent statistical analyses using GraphPad PRISM version 10.0 (GraphPad Software, Boston, USA). To determine if the data followed a normal distribution, a normality test was performed on each set of data using the Kolmogorov-Smirnov test. Non-parametric correlations were performed employing the Spearman coefficient. A p value of <0.05 (5%) was considered as statistically significant.

3. Results and discussion

Surface water area (km^2) for each of the 24 European countries is shown (Fig. 1). PA and NTM infection

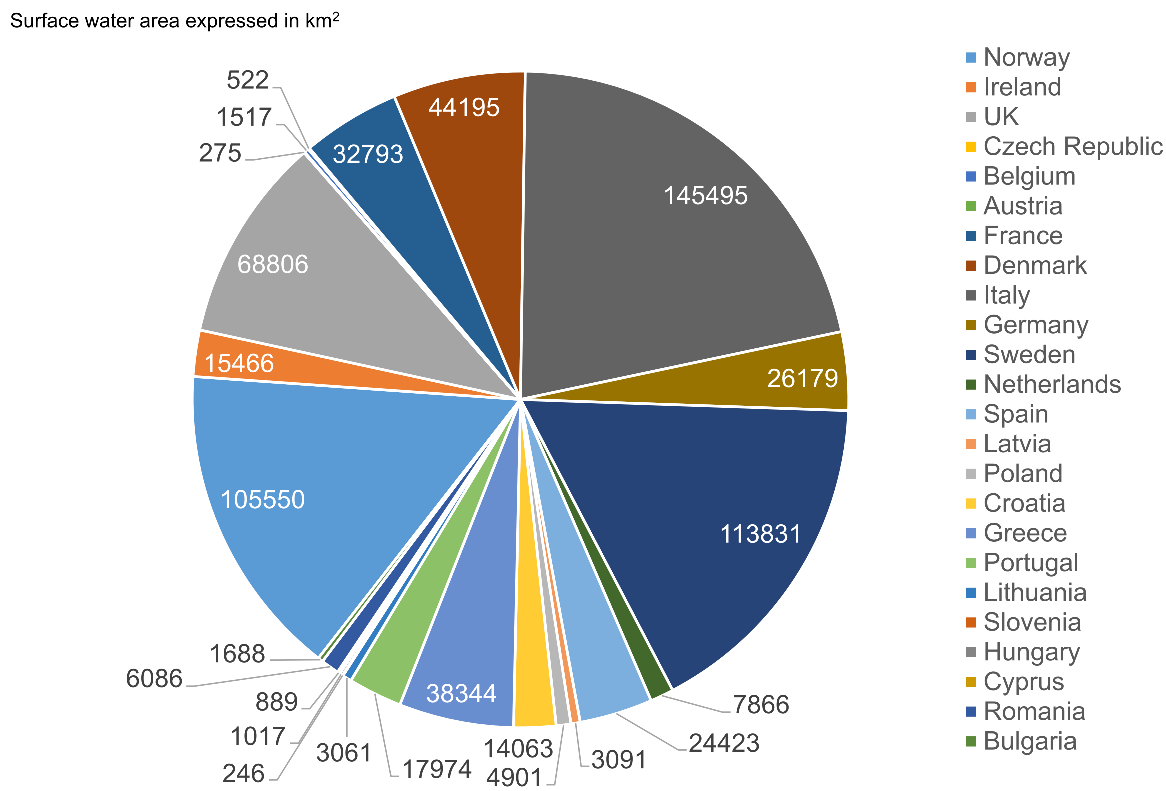

Fig. 1. Total area (km^2) of surface water in 24 European countries. Source of data: WISE- Freshwater. Freshwater Information System for Europe. https://water.europa.eu/freshwater/data-maps-and-tools/water-framework-directive-surface-water-data-products/delineation-surface-water.

rates in children and adults are shown (Figs. 2 and 3, respectively). The correlation between surface water area and PA and NTM infection for 24 European countries, is shown in Figs. 4 and 5, respectively.

Some controversies remain in this field, namely what is the source of *Pseudomonas aeruginosa* for the person with CF? How important a role does surface water play as a potential source of this orgamism in CF? Various publications have attempted to address these conundrums, including a seminal review on risk assessment of *Pseudomonas aeruginosa* in water [13]).

To our knowledge, this is the first study to examine the relationship between surface water area and infection rates in people with cystic fibrosis, across 24 European countries at a national level. This study made this correlation analysis based on 678,278 km^2 of surface water area with 41,486 CF patients in 24 European countries and showed that there was no correlation between any of these parameters (PA, NTMs, adults and children & surface water area), indicating that infection rates did not increase when the area of surface water increased, thereby disproving our original hypothesis that infection rates increase as surface water area increases, due to the probability of patient exposure and pathogen acquistion increasing with the surface area of waters.

In this study, PA and NTMs were selected as surrogate organisms to examine, as both are closely associated with water, as one of their natural environmental sources, as well as these bacteria being clinically significant in this patient population. Such surface water sources may be used for drinking, recreational and aesthetic purposes. Previous studies have examined the relationship between the infection rates of PA and NTM CF patients and have attempted to correlate these with the proximity of these patients to their nearest surface water. A study in Belgium by Goeminne and colleagues [9] examined 28 adult CF patients with no previous history of PA and compared these to 28 age and sex-matched controls who were chronically colonised with PA. For those with PA, the distance between their home and the closest blue space was calculated. Patients who were never infected with PA lived significantly further away from a natural water source than those chronically colonised with PA (mean 487 m vs 308 m, p = 0.014) and beeline (mean 324 m vs 202 m, p = 0.021). These authors concluded that natural open water represents a source of infection by PA in CF patients. Similarly, a study by Bouso and colleagues [14] of 65 CF children (n=21 NTM+ve; n=44 NTM-ve) in Florida in 2017, showed that children who lived within 500 metres of water were 9.4 times more likely to acquire NTM (p = 0.013).

Water, in its various forms, including surface water, river water, lake water, tapwater and bottled water, has been associated with the occurrence of PA [13]. This

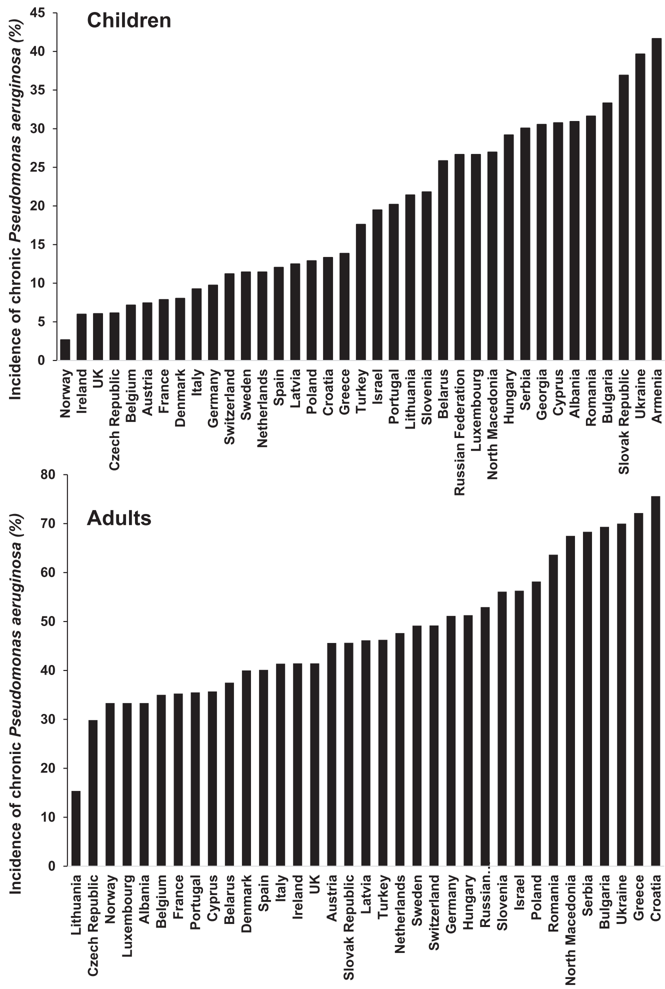

Fig. 2. Incidence of children and adults with cystic fibrosis with chronic *Pseudomonas aeruginosa* infection (%). Source of data: European Cystic Fibrosis Society. ECFS Patient Registry Annual Data Report 2019. https://www.ecfs.eu/sites/default/files/general-content-files/working-groups/ecfs-patient-registry/ECFSPR_Report_2019_v1_16Feb2022.pdf.

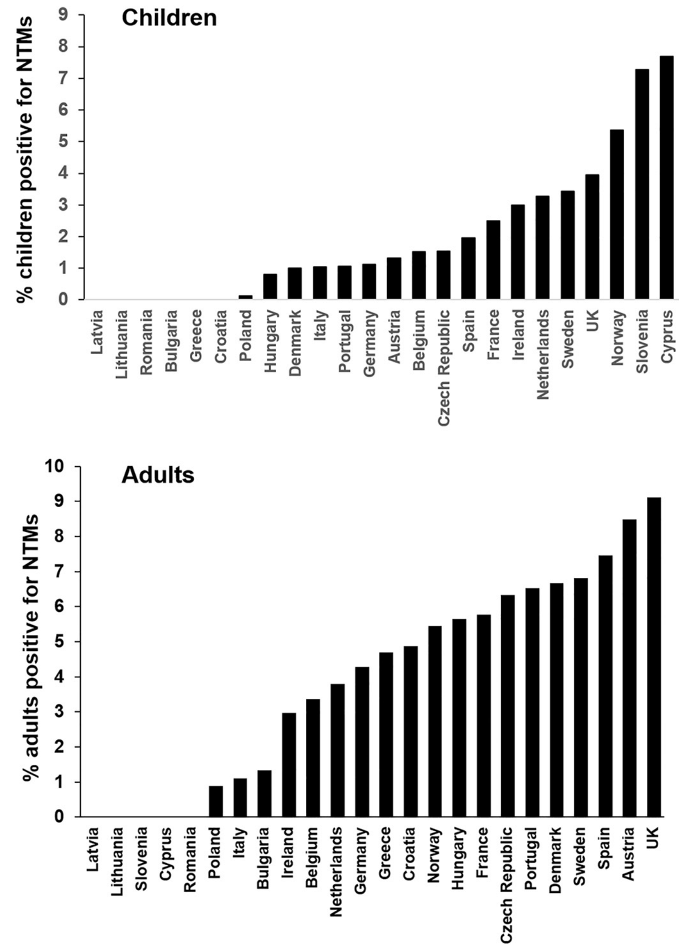

Fig. 3. Incidence of children and adults with cystic fibrosis with non-tuberculous mycobacteria (NTM) infection (%). Source of data: European Cystic Fibrosis Society. ECFS Patient Registry Annual Data Report 2019. https://www.ecfs.eu/sites/default/files/general-content-files/working-groups/ecfs-patient-registry/ECFSPR_Report_2019_v1_16Feb2022.pdf.

study hypothesised that if physical exposure to surface water was greater, then over time, there would be a greater risk of exposure to PA, manifesting in higher incidence rates of PA in people with CF. This was a high level study attempting to examine potential correlations between surface water area in European countries and incidence of PA in people with CF in the same countries, using paired data. However, we were unable to find such an association. Our hypothesis was that if there was an abundance of surface water, then there would be greater exposure by means of its physical presence, as well as associated factors involving surface water, such as water-related leisure activities, including fresh water swimming, water sports, rowing, boating, fishing and other recreational uses of water. Recent scientific data also highlights the variability and survival dynamics of PA in surface water, where abiotic and biotic factors interactively affect the decay of PA [15].

Acquistion of PA and its avoidance in people with CF is therefore a complicated dynamic, not solely driven by close association with surface water, but through the integration of several other factors, including mitigations by people with CF to avoid high risk scenarios with surface water. Further work is now required to help understand the quantum of risk avoidance in people with CF and surface water exposure.

3.1. Antimicrobial resistance (AMR) and water as a source of novel antimicrobials

Antimicrobial resistance (AMR) has now emerged as a critical global threat to public health [16]. The two bacterial pathogens discussed in the current study come from an enhanced pedigree of highly antibiotic resistant organisms. The consequence of such high level resistance in these organisms has profound clinical implications for the person with CF, including (i) difficulty in treating pulmonary exacerbations, (ii) the need to use several antibiotics concurrently and (iii) potential denial of clinical procedures, including lung transplantation. Whilst water

Table 1
Sources of novel bioactive antimicrobials from marine environments.

Name of antimicrobial substance	Name of producing organism	Source	Activity	References
N-demethyltyroscherin	*Scedosporium apiospermum* FKJ-0499	Deep-Sea sediment	Antifungal	[17]
Hatsusamides A and B	*Penicillium steckii* FKJ-0213	Deep-Sea-derived	Antimalarial	[18]
Macrolides	Several marine organisms (*Streptomyces* spp)	Marine environment	Antituberculous	[19]
Cladomarine	*Penicillium coralligerum* YK-247	Deep-Sea fungi	Anti-saprolegniasis	[20]
Quellenin	*Aspergillus* sp. YK-76	Deep-Sea fungus	Anti-saprolegniasis	[21]

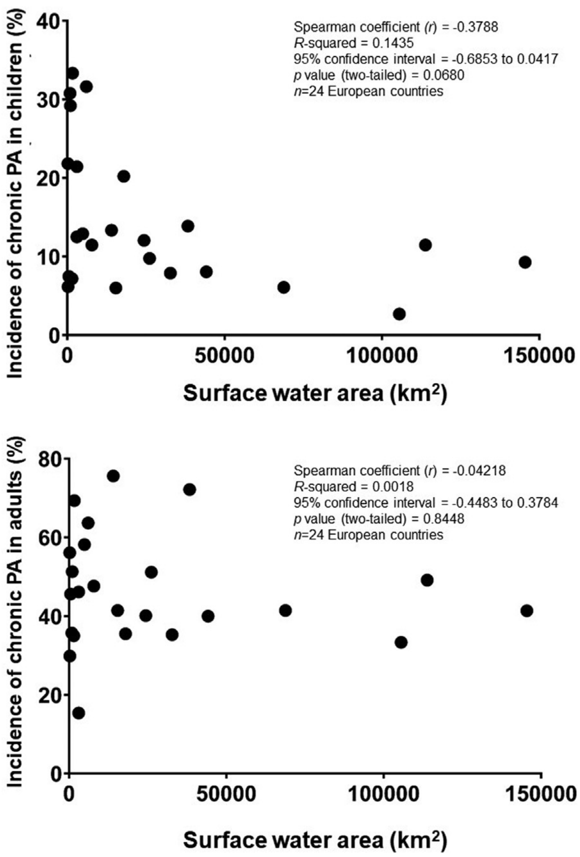

Fig. 4. Correlation employing Spearman coefficient between children and adults with cystic fibrosis with chronic *Pseudomonas aeruginosa* infection and surface water area.

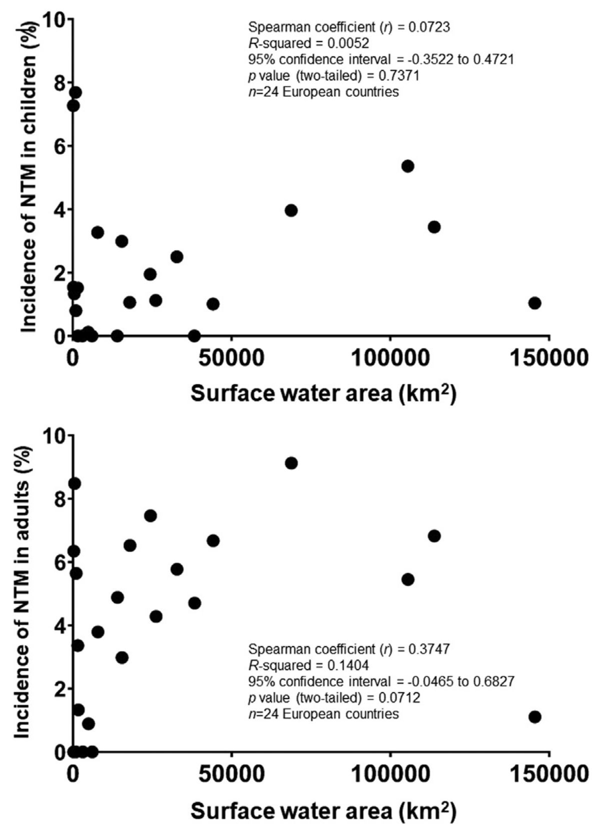

Fig. 5. Correlation employing Spearman coefficient between children and adults with cystic fibrosis fibrosis with non-tuberculous mycobacteria (NTM) infection and surface water area.

has been described as a source of microorganisms which can be antimicrobial resistant, it may paradoxically be the source of novel antimicrobial agents which can be employed to treat such infections. Several studies have reported on potential antimicrobial agents for bacteria, as detailed in Table 1 [22].

3.2. Limitations and future work

The study presented has several limitations. Very limited surveillance data is available that describes the occurrence of these organisms in such waters in all countries examined and whether or not there are geoclimatic factors that could support their survival in waters in different countries. There is also limited data to indicate the direct and indirect involvement of CF patients with these water sources. Furthermore, acquistion of these organisms in such patients is complex, involving several factors including patient CFTR mutation type and patient behaviour relating to cross infection and infection control. Given that water continues to be a well documented environmental source of these organisms, CF patients should continue to be vigilant about potential infection risks posed by water and take evidence-based decisions regarding their behaviour around water to protect them for acquiring these organisms from these sources.

Further studies involving larger patient cohorts in different geoclimates are required to establish the relationship between spatial distance to bluespaces and infection in CF patients with waterborne CF respiratory pathogens.

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Author contributions

John E. Moore: Conceptualization, Formal analysis, Methodology, Writing – original draft, Writing – review & editing. **Beverley C. Millar**: Conceptualization, Formal analysis, Methodology, Writing – original draft, Writing – review & editing.

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None.

Declaration of competing interest

The authors declare that the research was conducted in the absence of any commercial, financial or other relationships that could be construed as a potential conflict of interest.

Data available statement

Data sharing is not applicable to this article as no data sets were generated or analyzed during the current study.

Ethics statement

This study did not involve human or animal subjects. All of the material used in this study was openly and freely available to the public as cited and within the public domain.

Informed consent

Not applicable.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.imj.2024.100125.

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