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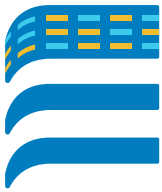
Brazilian Journal of Health and Biomedical Sciences

VOL. 24, Nº 1, JAN-JUN/2025



BJHBS

Brazilian Journal of Health and Biomedical Sciences

Vol. 24, número 1, janeiro-junho/2025

Rio de Janeiro

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Partially supported by

FAPERJ
Fundação Carlos Chagas Filho de Amparo à Pesquisa do Estado do Rio de Janeiro

Classified in

QUALIS

CATALOG AT SOURCE
UERJ/REDE SIRIUS/CBA

Brazilian Journal of Health and Biomedical Sciences. – V. 24, n. 1 (jan.-jun.2025) . – Rio de Janeiro: HUPE, 2002- v. : il. (some color.)

Semestral 2002-.
Available at: bjhbs.hupe.uerj.br
Previous title: Revista Hospital Universitário Pedro Ernesto.

1. Ciências médicas – Periódicos. 2. Saúde – Periódicos. I. Hospital Universitário Pedro Ernesto.

CDU 61

Librarian: Thais Ferreira Vieira - CRB - 5302

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The benevolent dictatorship: prejudice disguised as science.

Throughout history, it has become clear that morality often disguises itself as technology, and opinion masquerades as data. The consequences are often catastrophic. The "benevolent dictatorship" is a silent regime in which prejudice is authorized, as long as it is wrapped in supposedly neutral language, legitimized by reports, protocols and consensuses that tend to reproduce the same moralism they pretend to combat for the benefit of the collective. Thus, old prejudices can gain a new lease of life, supported by a pseudo-scientific authority that dismisses uncomfortable questions and avoids critical review.

This fact can be seen in some current criteria for biomedical treatments (Figure 1), body selection, or behaviors. Under the pretense of protecting the majority, an inconvenient minority is created, marked by statistical, decontextualized labels that have little to do with real individuals. No room can be found for historicity or consideration of social determinants: everything is resolved in the coldness of a list or the assertiveness of a protocol as absolute, doctrinal truth.

In such arrangements, science turns into a shield: it protects those who wield it, harms those who don't fit its mold. It becomes dogma when it should be a process; it becomes a statement when it should be a question. This is the science of Cartesian rigidity that upholds seemingly beneficial norms but perpetuates inequalities under the pretext of collective concern.

Ultimately, the "benevolent dictatorship" is simply a more polished way of controlling bodies and behaviors. And perhaps therein lies its greatest danger. It is developed in air-conditioned rooms, in legitimate meetings of associations, wielding sacred checklists. And thus, it stifles doubt—which should be science's greatest ally—in name of a supposed consensus. As if someone needs to have more common sense.

Science exists primarily because of the human capacity to ask questions, and the quality of these questions defines its importance. Paradoxically, science lends itself to upholding prejudice. We should not confuse technical prudence with a façade of moralism. If science itself fails to find the answer and materialize it, the "benevolent dictatorship" will continue, cordial and implacable, convinced of its rightness—and fairness.

Figure 1. Slovakia 2 Euro commemorative coin, 2023. 100th anniversary of the first blood transfusion. KM# 195.

Eloísio Alexsandro da Silva Ruellas
Editor In Chief

DOI: 10.12957/bjhbs.2023.80912

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Updates on the antimicrobial properties of garlic (*Allium sativum*) biomolecules in the treatment of human infectious diseases

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Abstract

Garlic (*Allium Sativum*) is extensively consumed as a seasoning and can be classified as a medicinal food due to its anti-inflammatory and antimicrobial properties. This study summarizes the garlic biomolecules and presents an update on garlic's main antimicrobial properties, correlating them with its phytochemical composition. This review article is based on a search for articles in the SCOPUS, PubMed, and SciELO databases, using ((anti-infective agents) OR (antimicrobial)) AND (garlic) descriptors. Garlic is a good option for association with traditional medicines and emerges as a potential source of phytochemicals. It has applications *in natura*, in aqueous and alcoholic extracts, and as essential oils. The macrostructure of garlic is divided into roots, bulb, stem, clove and leaves. Phenolic compounds and flavonoids, which are important constituents in its antimicrobial character, are found in the leaves. Allicin, garlic's main bioactive component, is mostly located in the stem and leaves. The effects of garlic influence the metabolism, the stabilization of the plasma membrane, the adhesion of microorganisms and the formation of biofilms by bacteria, mainly *Escherichia coli* and the fungus *Candida albicans*. This study concludes that *Allium sativum* is an option in the development of potential antimicrobial phytochemicals and an excellent contributor to the strengthening of the immune system.

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BJHBS, Rio de Janeiro, 2024;24(1):10-20
DOI: 10.12957/bjhbs.2024.85192
Received on 06/10/2024. Approved on 06/01/2025.

Keywords: Phytotherapy; antimicrobial activity; allicin; infection; bacteria; antibiotics; natural compounds.

Introduction

Antimicrobials – including antibiotics, antivirals, antifungals and antiparasitics – are medicines used in the prevention and treatment of infectious diseases in humans, animals and plants. Antimicrobial resistance (AMR) occurs when bacteria, viruses, fungi and parasites no longer respond to antimicrobial drugs. As a result of drug resistance, antibiotics and other antimicrobial drugs become ineffective and infections become difficult or impossible to treat, thus increasing the risk of the spread of diseases, serious illness, disability and death. AMR is a natural process that occurs over time through genetic changes in pathogens. Its emergence and diffusion are accelerated by human activity, especially by the misuse and overuse of anti-

microbials to treat, prevent or control infections in humans, animals and plants. Antimicrobial resistance (AMR) is a major global threat to public health and development. Bacterial AMR was directly responsible for an estimated 1.27 million global deaths in 2019 and contributed to 4.95 million deaths.[1]

AMR puts many of the gains of modern medicine at risk. It makes infections more difficult to treat and significantly increases the risks associated with other medical procedures and treatments. In addition to death and disability, AMR has significant economic costs. The World Bank estimates that AMR could result in US$1 trillion in additional healthcare costs by 2050 and losses of US$1 trillion to US$3.4 trillion in gross domestic product (GDP) per year by 2030[2]. Priorities for addressing AMR in human health include strategic innovation and the research and development of new medicines. In this regard, compounds extracted from plants show promise. Garlic has potential as an antimicrobial agent, and the dissemination of knowledge and research on this plant should be encouraged.

The use of plants to treat illnesses and promote overall well-being dates back thousands of years. Species such as *Zingiber officinale* (ginger), *Mystirica frangrans* (nutmeg) and *Allium sativum* (garlic) gained prominence due to their antimicrobial activity and are currently being studied as potential medicines to combat microorganisms.[3,4,5] However, it should be noted that the use of these species goes back to antiquity, as exemplified by *Allium sativum*, which was used by many people to treat diseases. Since that time, garlic has been credited with fungicidal and bactericidal properties, in addition to being considered useful in the treatment of heart disease, headaches, diabetes and even cancer.[6,7,8]

The *Allium sativum* species belongs to the Amaryllidaceae family, has Asian origins and is produced all over the world. It is composed of a subglobose bulb, divided between 620 bulbils (garlic cloves) surrounded and held together by protective leaves. The bulbils are attached to a stem, which has some fibrous roots on its lower surface. Each bulbil has an ovoid shape, is asymmetrical and has a whitish, pink, or violet color. In cross-section, the bulbil is formed by an outer part, called the scarious prophyll, which surrounds a reserve cataphyll, a fleshy structure, in which the main assets with vegetable medicinal properties are found.[3,9]

The search for alternative antimicrobial medicines, such as garlic, has gained importance in the current context, in light of the growth in bacterial resistance and the difficulty in combating pathogens that cause common infections by use of traditional antibiotics. This phenomenon is related to the misuse and abuse of these medications. In this sense, research into new antimicrobials can help in the search for effective treatment options against bacterial resistant infections.[10,11] Therefore, the main objective of this study is to provide an update on the antimicrobial properties of the species *Allium sativum*, through the compilation and critical analysis of available sources, in order to understand how this vegetable can influence the treatment of infectious diseases. Finally, we also seek to understand the varied chemical components that can be extracted from the different structures of the plant.

Materials and methods

A search was made for original articles on the PubMed, Scopus and Scielo platforms, following PRISMA guidelines (Reporting for Systematic Review and Meta-Analysis Protocols)[12] to guide the research. Two different reviewers, using pre-established standards, independently read articles that presented studies on the antimicrobial properties of garlic and reached a consensus on which ones would be selected and which ones excluded. The descriptors were identi-

fied using the MeSH terms (Medical Subject Headings) thesaurus and an advanced search was carried out using Boolean operators. Both platforms used a combination of descriptors: "Garlic AND Anti-infective agents" and "Garlic AND Antimicrobial".

Table 1. Search strategy for the literature review and number of results found in the databases

	PubMed	Scopus	Scielo
Identification (total)	67	306	67
(Garlic) AND (Anti-infective agent)	33	161	47
(Garlic) AND (Antimicrobial)	34	144	17

End date of search: 31/Jan/2024

Source: The authors (2024).

First, the titles and abstracts of the studies were read. Then, the texts selected in the first stage were read and a final selection of articles for review was made. The inclusion and exclusion criteria for the articles were discussed and agreed among the researchers.

Studies were excluded according to the following criteria: i) showing the properties of garlic, but not its antimicrobial properties; ii) showing plants' antimicrobial properties, but not those of garlic; iii) lack of an original study (reviews, editorials, comments, letters); iv) articles in languages other than Portuguese, English or Spanish. The following inclusion criteria were used: i) original articles (clinical trials, randomized clinical trials, controlled clinical trials and case reports); ii) demonstrated association of antimicrobial properties and garlic; iii) articles that analyzed the antimicrobial properties of garlic in humans, animals and *in vitro*; iv) articles in Portuguese, English or Spanish; and v) articles on garlic on its own were included in this research.

Table 2. Flow diagram of survey results, based on preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement

IDENTIFICATION	ARTICLES IDENTIFIED PubMed (67) Scielo (67) Scopus (306)	TOTAL 440
SCREENING	**440 articles**	**337 articles excluded.** 58 were duplicates. Garlic not associated with antimicrobial properties Lack of originality
ELEGIBILITY	**103 article titles or summaries read**	**57 articles excluded** 6 articles -> Garlic associated with antibiotics 5 articles -> Garlic associated with other therapies 20 articles -> Garlic in food 20 articles -> Garlic against oral pathogens Others
INCLUDED	**46 articles**	17

Source: The authors (2024).

After application of the criteria, 103 articles were selected. These were tabulated and classified as follows: garlic by itself (46 articles); garlic with antibiotics (6 articles); garlic with other substances (5 articles); garlic in food (20 articles); garlic against oral pathogens (20 articles); others (6 articles). Only articles solely about garlic are reviewed in this research.

Results

Initially, 440 articles were selected, but only 46 met the pre-established criteria and were eligible to take part in this review. The selected articles were reviewed, and some topics on *Allium sativum* (garlic) were found to be frequently addressed. These can be divided into the following categories: phytochemical composition of the extract; antimicrobial properties and sensitive pathogens; efficacy of garlic extract when compared to those of other plants; efficacy of garlic extract when compared to broad-spectrum antibiotics used in medical practice (Table 3).

Table 3. Subject, objective, methods and highlights extracted from the articles included in this study

Subject	Aim	Method	Highlights
Phytochemical composition[13-19]	Analysis of the phytochemical composition of *Allium sativum* in different regions of the world. Trials were carried out to identify and quantify the phenolic and organosulfur compounds, vitamins, minerals, and volatile and non-volatile substances present in garlic; in addition, the main active compounds in garlic were identified with regard to their antimicrobial and antioxidant properties, as well as the influence of processing methods on such properties.	The garlic was harvested in different parts of the world (e.g. Australia, Nigeria and Brazil). The plant was dried at room temperature or in the sun, crushed using a pestle or other instrument and then transformed into powder. From this, aqueous or alcoholic extracts were obtained, using techniques such as ultrasound, Soxhlet, Headspace SPME and maceration. The extracts were filtered for antimicrobial analysis. Techniques of diffusion in agar, microdilution in well or disk were then used. Finally, the minimum and maximum inhibitory concentration was determined for each microorganism tested and any growths were analyzed. The inhibition zones for each pathogen were measured. Three different methods were tested to observe the respective impact of these methods on the chemical composition of the garlic. Three different methods were tested: shade drying, freeze drying and microwave drying. In addition, the impact of roasting garlic on its biochemical properties was also evaluated.	Garlic has several bioactive compounds with antimicrobial properties. The most important component is allicin; however, several studies have emphasized the importance of y-glutamyl-S-allyl-cysteine, γ-glutamyl-phenylalanine and E- and Z-ajoenes. Ajoenes gained prominence in the study against pathogens involved in Tinea and showed good results, being a possible ally to terbinafine, the drug currently used against this condition. Thirty organosulfur compounds and 51 non-volatile compounds were also identified in garlic, including dipeptides, flavonoids and phenolic acids. Many studies still need to be carried out on substances that are unknown or have not been the subject of extensive study. In addition, with regard to drying methods, studies found that freeze drying, and microwave drying were the best ways to preserve the composition of garlic. The type of drying that had the least impact on antimicrobial potential was freeze drying. Finally, with regard to the roasting process, the results concluded that roasted garlic had better bioactive properties than garlic *in natura*.
Antimicrobial properties and sensitive pathogens[5,7,20-36]	Evaluation of garlic and its antimicrobial properties, with emphasis on determining which microorganisms are sensitive to the plant and at what concentration.	Pathogens collected from different sites (skin, mouth, vagina, prostheses, among others) were isolated and placed in specific culture media (Blood agar, Nutrient agar, Sabouraud agar) depending on the microorganism under evaluation. Subsequently, aqueous garlic extract, garlic essential oil or garlic *in natura* (depending on the study) were added in different concentrations using the microdilution technique in wells, agar diffusion or disc diffusion and determining the maximum and minimum inhibitory concentration. The plates were subsequently checked for the presence of any bacterial growth.	The studies found that various pathogens were sensitive to garlic extract, which effect was related to the concentration of the extract. For this reason, the garlic extracts were tested at different concentrations to assess their antimicrobial capacity. At a concentration of 25mg/mL, none of the pathogens evaluated - *Staphylococcus aureus*, *Escherichia coli* and *Klebsiella pneumoniae* - were sensitive. At a concentration of 50mg/mL only *E. coli* was sensitive, showing an inhibition zone of 5cm. At a concentration of 75mg/mL, a zone of inhibition measuring 8cm was observed for *S. aureus* using the aqueous extract (EA) and 10cm using the alcoholic extract (EAL), a zone of inhibition for *E. coli* of 9cm (EA) and 10cm (EAL) and a zone of inhibition for *K. pneumoniae* of 8cm (EA) and 7cm (EAL). In this test, all the pathogens proved to be sensitive, but some studies have questioned the sensitivity of *E. coli*, which has proven to be resistant in other experiments. Furthermore, garlic has shown antifungal potential against *Candida* spp. and *Sporothrix schenckii*. Studies have also tested garlic's ability

Source: The authors (2024).

Table 3. Subject, objective, methods and highlights extracted from the articles included in this study (cont.)

Subject	Aim	Method	Highlights
Antimicrobial properties and sensitive pathogens[5,7,20-36]			to inhibit fungi that cause Tinea. Ointments containing the extract were used and the results were promising, but more studies are needed on this topic. The anti-*Giardia* capacity of garlic extracts was also evaluated, and garlic was shown to be a potential medicine for use against this protozoan, since allicin has the capacity to inhibit the pathogen.
Comparison with other antibiotics[34-44]	Comparison of the antimicrobial effects of *Allium sativum* with those of traditional medicines already used to treat infections. Antibiotic and antifungal drugs were analyzed against a variety of pathogens common in clinical practice. The studies varied in the way the plant was analyzed, with the majority using the aqueous extract of the plant. The degrees of inhibition, response time and doses required for each therapy were compared, with most studies seeking to find the Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal/Fungicidal Concentration (MBC) for garlic and the drugs according to each pathogen analyzed.	Most of the studies analyzed the minimum concentrations using the agar diffusion method. Samples of bacteria and fungi were isolated from various places, such as vaginal lesions, the nasopharynx of infected people, beef, urine, among others. After isolation, each pathogen was cultivated in the appropriate medium for its growth, respecting the incubation time required for the analysis to be carried out. Meanwhile, garlic was prepared for addition to the microorganisms' culture medium. Most of the studies used the plant's aqueous extract, made by mixing a certain amount of the plant in grams with a few mL of distilled water. Another group of studies used fresh garlic, cut into discs and applied directly to the pathogens. In the case of the isolated analysis of certain garlic compounds, the extraction processes used materials such as methanol. Subsequently, the garlic samples and traditional medicines were added to the microorganism isolates by diffusion methods in Petri dishes or by microdilution in test tubes. Finally, the macroscopic aspects of the result were observed, such as the appearance of turbidity and the measurement of the diameter of the inhibition halos. Microscopic characteristics were analyzed using electron microscopy, spectrophotometry and colorimetry methods. Most of the analysis used a simultaneous negative control.	Most studies have shown that garlic has similar or even greater antimicrobial potential than some traditional medications. In studies comparing the effects of the plant with the efficacy of fluconazole on *Candida* spp. isolates, the combination of two garlic extracts proved to be more effective than the drug in inhibiting the growth of the pathogen. In another study, resistant strains of some microorganisms, such as *Methicilin Resistant Staphylococcus aureus* (MRSA) and *Pseudomonas aeruginosa*, also proved resistant to various concentrations of garlic extracts. The plant's main mechanisms of action include destabilizing the plasma membrane and interfering with the metabolism of pathogens. Much of the research suggests that the plant has the potential to be combined with traditional medicines to enhance the treatment of infections in the community and in health centers. However, it is important to emphasize that most of the studies have been carried out using *in vitro* tests and more *in vivo* tests need to be conducted in order to draw more solid conclusions about the efficacy and safety of using garlic.
Comparison with other plants[28,39,45-53]	Comparison of the antimicrobial effects of *Allium sativum* with other plant varieties and other natural products.	The main compounds of each plant or natural product were extracted. Different methods were used in the varieties of studies analyzed. Garlic compounds were extracted under pressure and with the addition of an extractant, such as distilled water, methanol or physiological NaCl solution. Their antimicrobial properties were analyzed using the diffusion method, with subsequent calculation of the zone of inhibition around the site where the extract was applied. The efficacy of extracts obtained from garlic was compared with those of other varieties, such as ginger, chili, lemon and honey. Each study selected a specific pathogen, for example, *Streptococcus mutans, Escherichia coli*, among others.	The comparison of the antimicrobial activities of garlic species with those of other plants, showed that *Allium sativum* was much more effective. In comparative studies that also analyzed lemon, honey and ginger, garlic showed a potent anti-infective activity against a greater number of pathogens and faster action in improving symptoms and remission of the disease. In other studies, which also evaluated the properties of some onion species, garlic showed efficacy against almost all pathogens, while the other plants showed little or no effect. It should be noted that a large part of garlic's antimicrobial character is derived from the presence of allicin and phenolic compounds in its composition. Finally, all the studies highlight the need for further analysis to consolidate and innovate knowledge about the use of garlic and other plants in the treatment of infections.

Source: The authors (2024).

Discussion

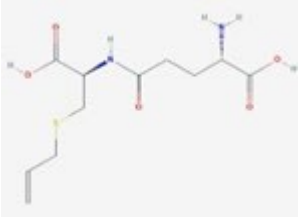
Antimicrobial resistance is a public health problem, the solution of which depends on the search for therapeutic alternatives for treatment of resistant microorganisms. Among the promising alternatives, pharmacological repositioning and research on the development of new drugs stand out. Research into the pharmacological potential of plants is one possible avenue of action and has been gaining prominence in recent years. Since garlic is a food with pharmacological properties that are already well-known to many civilizations, scientific experimentation to evaluate therapeutic evidence is essential. This study aims to gather and discuss the results of research on the antimicrobial potential of garlic to facilitate new studies based on the existing scientific evidence.

Contemporary studies have confirmed the importance of the medicinal properties of this plant in the context of infectious diseases, due to its antibacterial, antifungal and antiviral characteristics. Much of this function is known to be derived from the organosulfur compounds, flavonoids and terpenoids present in garlic. [8,45] Recent research analyzed the inhibitory potential of the vegetable on the growth of microorganisms that cause recurrent infections in the community and in hospital contexts. Several studies have already demonstrated the action of *Allium sativum* in controlling *Escherichia coli*, *Candida albicans* and *Streptococcus* spp.[14,15,16] Furthermore, an ability to inhibit the growth of viruses such as *Coxsackievirus* spp. and herpes simplex virus types 1 and 2 was also observed.[47]

The phytochemical composition of the extract made from garlic cloves was found to be rich in bioactive compounds that enhance its medicinal and nutritional properties. The main active compounds in garlic are organosulfur constituents, such as allicin, which have different mechanisms of action related to fighting infections. They are responsible for many of garlic's health benefits, including antioxidant and antibacterial activity. In this regard, these components can prevent the formation of biofilm by bacteria, destabilize the plasma membrane of microorganisms and interfere with the synthesis of DNA and RNA, in addition to other processes that act on the different forms of growth and survival of pathogens.[3,4]

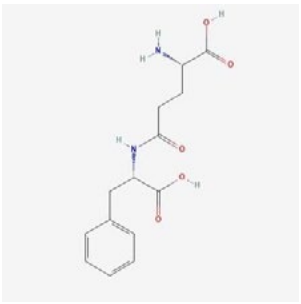
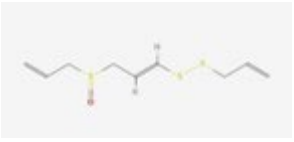
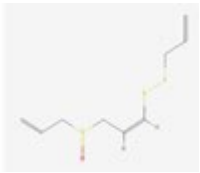
According to Torres *et al.*[15] and Barbu *et al.*,[14] other compounds with antibacterial function are y--glutamyl-S-allyl-cysteine, γ-glutamyl-phenylalanine and E- and Z-ajoenes. Garlic also contains phenolic compounds, such as flavonoids and phenolic acids. Other important substances include vitamins, especially vitamin C, and minerals, such as selenium and magnesium.

Table 4. List and structure of some compounds isolated from Allium Sativum

Compounds	Molecular formula	Structure
Allicin	C6H10OS2	
Gama-glutamyl-S allycisteine	C11H8N2O5S	

Source: https://pubchem.ncbi.nlm.nih.gov/

Table 4. List and structure of some compounds isolated from Allium Sativum (cont.)

Compounds	Molecular formula	Structure
Gama-glutamyl-phenylalanine	C14H18N2O5	
E-ajoene	C9H14OS3	
Z-ajoene	C9H14OS3	

Source: https://pubchem.ncbi.nlm.nih.gov/

In line with this finding, another study[45] detailed the phytochemical composition of five parts of the food that are usually wasted – the root, garlic clove skin, garlic peel, flower stalk and leaf – and quantified the phenolic compounds and flavonoids present in them and their antioxidant capacity. The article concluded that the garlic leaf had an even higher content of phenolic compounds and flavonoids than the garlic clove and the other parts, as well as having an effective antioxidant and antitumor power. Allicin was found in significantly lower quantities in the flower stalk and leaves than in the garlic clove.

Research has been conducted to test the antimicrobial capacity of the aqueous/alcoholic extract of garlic against gram-positive and gram-negative bacteria and fungi. The main pathogens tested were *Escherichia coli*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Candida* spp.[14,35,45]

Vargas *et al.*[48] analyzed garlic extracts with different concentrations of allicin and concluded that a garlic extract containing 3mL of allicin was able to completely inhibit the growth of the bacteria *Staphylococcus aureus* and *Pseudomonas aeruginosa* and was unable to inhibit the growth of *Escherichia coli*, with this pathogen being more resistant to the antimicrobial compound studied at this concentration.

The other concentrations studied were unable to prevent bacterial multiplication. From a similar perspective, Abidullah *et al.*[49] also tested the efficacy of the aqueous and alcoholic extract of garlic against *Staphylococcus aureus*, *Klebsiella pneumoniae* and *Escherichia coli* at concentrations of 100mg/mL and observed the formation of an inhibition zone of 12mm (*S. aureus*), 7mm (*K. pneumoniae*) and 10mm (*E. coli*). It was therefore concluded that the extract was effective against these micro-organisms and that, at higher concentrations, *E. coli*

is sensitive to garlic. Finally, the antifungal potential of garlic, which was proven in a clinical trial[23] carried out with women diagnosed with candidiasis, is also worth highlighting. The women were given garlic tablets to treat the fungus, and the results showed that garlic could be a possible alternative to fluconazole, currently the gold standard drug for treatment of this condition. However, further studies are needed.

In general, garlic has greater antimicrobial potential than other vegetables when comparisons are made. Farbman *et al.*[50] evaluated the in vitro activity of garlic and onion species against a variety of bacteria and concluded that garlic is more effective, emphasizing the importance of components such as allicin. Onions did not exhibit any inhibition of the variety of pathogens tested. In addition, when studying the antimicrobial properties of a range of foods used in Indian cuisine (garlic, chilies, ginger, onions), Indu *et al.*[5] concluded that garlic was effective against all the bacteria investigated, such as *E. coli* and *L. monocytogenes*. The other vegetables, on the other hand, proved to be less or not at all effective. These conclusions demonstrate the need to devote more resources and studies to the properties of garlic, since it is one of the vegetables with the greatest antimicrobial potential known today.

Finally, it is worth noting that many authors have also evaluated the antimicrobial properties of the plant in comparison with traditional medicines. Ebrahimy *et al.*[44] compared the effects of garlic capsules in the treatment of *Candida* spp. vaginitis with the commonly used drug fluconazole, finding no significant differences in the response to treatment following the two interventions. Indu *et al.*[5] compared the activity of the plant with the performance of traditional antibiotics, such as ciprofloxacin and chloramphenicol, and the plant showed excellent antimicrobial activity and could be an alternative treatment in light of the increasing resistance and development of new strains of pathogens. Another study evaluated the antimicrobial potential of allicin against *T. rubrum*, the fungus that causes dermatophytosis, comparing treatment with ketoconazole. The study concluded that the concentration of allicin required to have the same effect as ketoconazole is higher, but that one substance is almost as effective as the other, and called for more in vivo experiments to advance knowledge of these healing properties of garlic.[24] These conclusions confirm the plant's potential to become an alternative or an ally to traditional treatments, thus helping to expand therapeutic approaches to community illnesses.

Conclusion

The studies analyzed show that *Allium sativum* is a plant with great antimicrobial potential, and appears to be a good option for association with traditional medicines or, possibly, replacement of them in the treatment of important conditions, such as infections by *Eschericia Coli*, *Staphylococcous aureus*, *Klebisiella pneumoniae*, *Pseudomonas aeruginosa* and *Candida* spp. This anti-infective property is derived from a variety of plant components, especially allicin and phenolic compounds. The studies evaluated the effects of garlic prepared in different ways, such as *in natura*, in aqueous and alcoholic extracts, as essential oils and in other forms.

One of the issues analyzed during the search for studies was the macrostructure of garlic, its divisions, and their possible relation with its properties. In this sense, the plant is divided into roots, bulb, stem, clove and leaves. The bulb is formed by the garlic clove, a fleshy structure, which is covered by a film. The largest number of phenolic compounds and flavonoids, which are important constituents in the antimicrobial character of the vegetable, were

found in the leaf. Meanwhile, allicin, the main bioactive component of the plant, is mostly located in the stem and leaves.

Among several conclusions, we can highlight the effects of garlic on the metabolism, in the destabilization of the plasma membrane of pathogens and in the adhesion of microorganisms, in addition to its influence on the formation of biofilms by bacteria. In this context, the action of the vegetable against the bacteria *Escherichia coli* and the fungus *Candida albicans*, which are relevant microorganisms in the treatment of patients in intensive care units, was deemed to be effective.

Based on all the knowledge acquired and considering the effectiveness of garlic, as well as the safety and the growth of microbial resistance to traditional medicines, one can conclude that *Allium sativum* presents a possible option in the treatment of patients and an excellent contributor to the strengthening of the human immune system. The vegetable appears to be an accessible and promising alternative and requires further studies to develop effective and reliable treatments for the best-known pathogens. The present study is an instrument to consolidate this knowledge and to promote new research on this subject.

Acknowledgments

The authors are thankful to Federal University of Viçosa for the opportunity to join a scientific initiation and write this article. We would also like to thank the faculty members Marli Cupertino, Rodrigo Batista-Siqueira, and Adriano Castro for their technical assistance and support during the development of this work. Lastly, we wish to thank our colleagues Gustavo Gomes and Gabriel Barboza. Their contributions were invaluable to the success of this project.

Declaration of interest statement

No potential conflict of interest was reported by the authors.

Data availability statement

The datasets generated used and analyzed during the current study are available from the corresponding author upon reasonable request.

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Analysis of the occurrence of the mesiobuccal canal in maxillary first molars using cone-beam computed tomography in a Brazilian population

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Abstract

Introduction: Although the success rate of endodontic treatment in molars can reach up to 91.7%, failures may occur due to the anatomical complexity of the canals and the presence of undetected canals, with the second mesiobuccal canal being the most frequently overlooked. Objectives: To assess the morphology of the first upper molar and the incidence of the second mesiobuccal canal using cone-beam computed tomography. Materials and Methods: Retrospective secondary data were collected from patients at a reference radiology clinic in Maringá, Paraná, and then underwent imaging exams with the Prexion 3D tomography machine between December 2015 and May 2016. Results: A total of 174 patients and 221 first upper molars were analyzed, with a higher prevalence of three roots (93%) and the presence of the second mesiobuccal canal in 57.4% of the teeth. The most frequent Vertucci classification was Type IV (38%). Conclusion: The study concluded that the first upper molar tends to have three roots and that the second mesiobuccal canal is present in more than half of the studied population, while the most common classification for this canal was Type IV.

Keywords: Second Mesiobuccal; Molar Tooth; Cone Bean Computed Tomography.

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BJHBS, Rio de Janeiro, 2025;24(1):21-26
DOI: 10.12957/bjhbs.2024.85197
Received on 29/12/2024. Approved on 25/04/2025.

Introduction

The root anatomy and canal morphology of upper molars have been extensively studied due to their complexity.[1] Published evidence indicates that most upper molars have three roots and four canals. Studies show that approximately half of the mesiobuccal (MB) roots contain a second canal, referred to as mesiobuccal 2 (MB2).[1,2]

The success rate of endodontic treatment in molars can reach up to 91.7%. However, despite the best efforts of clinicians, failures in treatment may still occur.[3,4] The potential reasons for such failures include the root canal's complex anatomy and undetected canals.[4,5] In their study, Mashyakhy *et al.*[6] found an 18% prevalence (30 out of 165) of undetected canals in teeth that had already undergone endodontic treatment, with the highest prevalence observed in

the first upper molars (40.6%). The second mesiobuccal (MB2) was the most frequently missed canal during the procedure.

Despite the existence of several studies on the incidence and prevalence of the MB2 canal, the available research reveals significant variation in the data. In the literature, the percentage frequency of the MB2 canal in upper molars ranges from 10% to 95%, depending not only on the method used in the study, such as radiography, scanning electron microscopy, micro-CT or cone beam computed tomography (CBCT)[7–9], but also on the ethnic and demographic composition of the population.[8,10] Kewalramani *et al.*[11] investigated the prevalence of the second mesiobuccal (MB2) canal in the first upper molars using CBCT images in an Indian population. The authors found a prevalence of 61.9% for the MB2 canal with three roots in the first upper molars. Similarly, Onn *et al.*[12] studied the prevalence of the MB2 canal in the first and second upper molars in a Bruneian population, finding a prevalence of 51.3% and 29.8%, respectively.

On the other hand, Lee *et al.*,[13] in a study conducted with a population from South Korea, identified a prevalence of 86.8% of the MB2 canal in the first upper molars and 28.9% in the second upper molars. In the study by Alnowailaty *et al.*,[14] conducted with a population from Saudi Arabia, the prevalence of the MB2 canal was 46.7% in the first upper molars and 17.7% in the second upper molars.

Given the variability in the prevalence of the MB2 canal in different populations, this study aims to assess and visualize, using cone beam computed tomography, the morphology of the first upper molar and the incidence of the second mesiobuccal (MB2) canal in a Brazilian population from a city in the state of Paraná.

Materials And Methods

Sample

This is a retrospective study based on secondary data collected from cone beam computed tomography images used for diagnostic purposes and treatment planning, obtained from patients treated at a reference clinic (Martinhão) in the northern region of Paraná. The clinic is equipped with a high-precision device, the Prexion 3D scanner (Prexion Inc, San Mateo, CA), which is essential for visualizing intricate details. Data was collected over a period of six months, from December 2015 to May 2016.

Inclusion and Exclusion Criteria

Inclusion Criteria: (a) presence of upper molars; (b) upper molars with fully formed apices; (c) absence of endodontic treatment.

Exclusion Criteria: (a) presence of intracanal posts; (b) presence of fixed prostheses; (c) teeth with internal and/or external resorption; (d) atretic teeth with no canal lumen; (e) residual roots; (f) presence of root fracture.

Tomographic Acquisition

The images were obtained using a small-volume cone beam computed tomography device, model Prexion 3D (Prexion Inc, San Mateo, CA). This device enables the visualization of small details, such as the fourth canal, which makes it eminently suitable for the proposed purpose.[15]

The initial specifications for the device used were operation at 90kV and 4.0mA; exposure time of 33.5 seconds; voxel size of 0.11mm; and a field of view (FOV) of 5.6cm x 5.2cm. It should be noted that none of the scans involved the use of contrast, and all tomographic exams were performed by an experienced technician under the supervision of a responsible radiologist.

Tomographic Evaluation

The images were analyzed dynamically in the axial, sagittal and coronal slices (Appendix I). The reading of the computed tomography images was performed using the CS 3D Imaging Software, on a Dell LCD screen with a resolution of 1920x1080 pixels, in a dark room, by two examiners who were previously calibrated and evaluated simultaneously.

Classification of Canal Morphology

The classification used was that of Vertucci (1984), as outlined in Table 1 below.

Table 1. Vertucci classification

Classification	Description
I	A single canal extends from the pulp chamber to the apex.
II	Two separate canals that join in the apical third.
III	One canal that divides into two, then reunites as one.
IV	Two separate and distinct canals until the apex.
V	One canal that divides just below the apex.
VI	Two canals that join in the root and then divide at the apex.
VII	One canal that divides, reunites, and exits through two foramina.
VIII	Three separate canals in one root.

Source: Vertucci (1984).

Since the Vertucci classification does not cover all teeth, an adaptation was made by adding the following classifications (Table 2):

Table 2. Adapted Vertucci classification

Classification	Description
IX	One canal that soon separates and ends separately.
X	Two canals, one independent and the other beginning below the chamber.
XI	Three canals, one independent and two separates that join near the apex.
XII	Two canals that join in the middle third.

Source: The authors (2024).

Data Analysis

The data were tabulated in Microsoft Excel 2010 spreadsheets (Microsoft Corp., USA) and analyzed descriptively with regard to their variables, such as age, gender, presence of the mesiobuccal canal, and classified according to their morphology.

Ethical Aspects

The study was approved by the Permanent Ethics Committee for Research Involving Human Beings (COPEP) of the State University of Maringá (UEM). (CAAE: 56129616.6.0000.0104 / Ethical Opinion: 1613490/2016).

Results

A total of 174 patients were included in the study, of which 112 (64%) were female and 62 (36%) were male. During the analysis, 221 first upper molars were evaluated, with 110 (49.77%) corresponding to the right first upper molar and 111 (50.22%) to the left first upper molar.

Among the 221 molars analyzed, 207 (93.6%) had three roots, while 14 (6.3%) had only two roots. In addition, of the total molars evaluated, 127 (57.4%) contained a mesiobuccal canal.

In the adapted classification, of the 127 molars that presented the fourth canal, 48 (38%) had a type IV classification, 28 (22%) had a type XII classification, and 19 (15%) had a type II classification (Table 3).

Table 3. Distribution of *n* and % of individuals with the fourth canal according to the adapted Vertucci classification, Maringá-PR, 2016.

VC*	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	Total
N	-	19	7	48	6	5	4	3	-	6	1	28	127
%	-	15	6	38	5	4	3	2	-	5	1	22	100

Legend: VC* - Adapted Vertucci Classification.
Source: The authors (2024).

Discussion

A thorough understanding of root canal morphology and its variations is essential for the success of endodontic treatment.[15,16] Studies indicate that the prevalence of a second mesiobuccal canal (MB2) in upper molars exceeds 50%.[15,17,18] This study aimed to evaluate the prevalence of the second mesiobuccal canal in the first upper molars of a Brazilian population from northern Paraná, using cone beam computed tomography (CBCT) as the detection method. The CBCT technique revealed that 57.4% of the analyzed molars had second mesiobuccal canals (MB2). Regarding the number of roots, 93.6% of the first molars examined had three roots, with the Vertucci type IV classification being the most common.

The literature demonstrates that the prevalence of the second mesiobuccal canal (MB2) ranges from 10% to 95%, depending on the method of analysis used and the demographic characteristics of the sample under study.[7-9] Previous studies, such as those by Kewalramani *et al.*[11] — which found a prevalence of 61.9% in an Indian population — and Onn *et al.*[12] — which found 51.3% and 29.8% for first and second upper molars, respectively, in a population in Brunei —

show that the occurrence of the MB2 canal can vary substantially among different population groups, which highlights the importance of conducting region- and ethnicity-specific studies.

The broad variation in the prevalence of the MB2 canal observed in the literature suggests the influence not only of genetic and ethnic factors but also of the detection method employed. CBCT stands out for providing an effective evaluation of root canal morphology due to its short exposure time, high resolution and accuracy, minimal distortion, three-dimensional visualization, and non-invasive nature.[18,19] In this context, the use of CBCT was crucial in capturing anatomical details with high precision, facilitating the identification of complex canals, such as MB2, with high reliability.

From a clinical perspective, the correct identification of the MB2 canal has direct implications for the success of endodontic treatments. Studies, such as that by Mashyakhy *et al.*,[6] indicate that failure to detect and treat additional root canals is one of the leading causes of the failure of endodontic treatments, with an undetected canal prevalence of 40.6% in upper first molars.[6] This data underscores the importance of using CBCT to enhance diagnosis and treatment accuracy.

In this context, the findings of Blank-Gonçalves *et al.*[20] — who reported a high prevalence of the MB2 canal in 90% of cases — further support the relevance of advanced CBCT imaging protocols. This notably higher detection rate, when compared to other studies, may be attributed to the refined acquisition parameters employed, including a small field of view (FOV) of 5×5cm and a voxel size of 0.085mm. These factors significantly enhanced both image resolution and the ability to detect complex anatomical structures, such as the MB2 canal and its variations.

Regarding the number of roots, 93.6% of the first molars analyzed in this study presented three roots, a result consistent with the study by Mheiri *et al.*,[21] which identified 98.9% of molars with three roots in an Emirati population. Supporting these findings, the study by Lin *et al.*,[19] conducted in a Taiwanese population, found that among the first upper molars examined, three (1.5%) had a single root, two (1.0%) had two roots, and 191 (97.5%) had three distinct roots. On the other hand, analysis of the study by Dibaji *et al.*,[22] showed that, of a total of 311 first upper molars evaluated, 153 (49.1%) had three canals, and 158 (50.8%) had four canals.

The Vertucci technique was an important study that focused on analysis of the internal anatomy of both upper and lower teeth to describe the root canal system (Vertucci, 1984). In his findings, Vertucci demonstrated that 55% of the first upper molars in an in vitro study had a second mesiobuccal canal, also referred to as the fourth canal, in the mesiobuccal root. The technique used for identification was diaphanization, which involved decalcifying the teeth and then staining them with a dye. The results found by Vertucci are consistent with the findings of the present study, although different techniques were used on in vitro teeth. In the current study, the morphology of the mesiobuccal root of the first upper molar, when the second mesiobuccal canal was present, showed the highest occurrence of classification type IV (38%), followed by type XII (22%) and type II (15%). On the other hand, Ratanajirasut et al.,[16] in their assessment of a Thai population, found that the presence of the mesiobuccal canal was most frequent in type I (36.4%), followed by type II (28.8%) and type IV (25.3%). In contrast, a study by Al-Saedi *et al.*,[23] conducted in an Iraqi population, showed that the occurrence of the second mesiobuccal canal was most prevalent in type II, at 44.58%.

The study concluded that the upper first molar has a high prevalence of three roots. The occurrence of the mesiobuccal canal was found in more than half of the population under study. The Vertucci Class IV classification was the most frequent for the mesiobuccal canal. Despite

the limitations of this study, the results contribute to the anatomical knowledge specific to the Brazilian population.

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BJHBS

Interictal balance changes in migraine - A stabilometric and diffusion tensor imaging study

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Abstract

Cerebellar dysfunctions have been found in migraineurs, while ischemic lesions have been described to be more frequent in their posterior fossa. To examine balance abnormalities and anatomical cerebellar changes in migraine interictally, 48 patients (21 with aura — MWA; 27 without aura — MWoA) underwent an evaluation of their stance by computerized static stabilometry (CSS) and were compared with controls. The frequency and amplitude of swaying in both the anteroposterior and latero-lateral axes, as well as the area and velocity of oscillations were estimated with open and closed eyes. A subgroup of 10 individuals and 10 controls was also examined with MRI and diffusion tensor imaging. Fraction anisotropy (FA) was obtained in nine regions of interest at the posterior fossa. Clinical parameters (age, age at onset, timespan of disease and frequency of attacks) were correlated with FA and CSS data. Subclinical impairment with greater lateral axis oscillation, especially in MWA, was observed. MWA patients were more dependent on visual input to control lateral sway than MWoA subjects. The anatomy of the cerebellum, especially at the dentate nuclei and middle cerebellar peduncles was comparatively impaired in migraine sufferers, as estimated by FA.

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BJHBS, Rio de Janeiro, 2025;24(1):27-37
DOI: 10.12957/bjhbs.2024.85381
Received on 28/05/2025. Approved on 16/06/2025.

Keywords: Migraine, Cerebellum, Stabilometry, Magnetic resonance, Diffusion Tensor Imaging.

Introduction

Migraine is a prevalent, paroxysmal, genetically driven, primarily neurological disease1 that compromises neurophysiology of a temporary or permanent nature. Although the most frequent and vigorous neurological symptoms of migraines, including headaches, occur during attacks, a series of interictal dysfunctions,[2] as well as possible structural changes,[3-5] have been reported.

Migraine may induce cerebellar and vestibular symptoms.[6] Around 2/3 of migraineurs are sensitive to motion and ¼ may present paroxysmal vertigo.[7] Migraine-associated dizziness has been correlated with vestibular dysfunction;[8] and a positive history of motion sickness with vestibular symptoms is relatively more prevalent in migraineurs.[9] Vestibular function

and balance are relatively more susceptible to visual influence in those migraine patients[10] whose the spatiotemporal function and visual motion processing are reported to be interictally abnormal.[11] Since the visual processing of migraine patients may be permanently impaired, it remains to be determined whether visual inputs play a role in some coordination and stance abnormalities associated with migraine. The cerebellum is particularly affected by infarct-like lesions in migraine patients,[5] which may theoretically lead to functional impairments. Hypermetria has been documented during finger-to-nose movements in migraineurs,[12] and stabilometric studies point to abnormal body sway in migraine.[13,14]

Fraction anisotropy (FA), a diffusion tensor imaging (DTI) magnetic resonance parameter that reflects the directionality of water diffusion due to cellular and subcellular components, may indicate the presence of lesions or dysfunctional tissues.[15] To further investigate migraine-induced cerebellar/vestibular abnormalities, the objective of the present study was to analyze interictal stance in migraine patients using Computerized Static Stabilometry (CSS) and correlate both balance and clinical parameters with fractional anisotropy (FA) values in cerebellar structures.

Methodology and resources

Twenty-one migraine patients with aura and 27 without aura who had been examined by neurologists experienced in the treatment of headaches, between April 2007 and July 2008, and diagnosed according to the 2004 International Headache Society criteria,16 randomly volunteered to undergo a CSS protocol between attacks. This procedure was also performed in 48 headache-free gender- and age-matched controls (HMC). Among these, 10 patients randomly selected and 10 matched controls were submitted to MR imaging. The age, age at onset, intensity of attacks as graded by the subjects from 1 (does not interfere with routine activity) to 4 (precludes activity since it is the most intense pain possible), frequency of attacks (as estimated by the subject), and timespan of disease in years were recorded for every subject. Inclusion criteria included: age between 18-65 years; presence of migraine for at least one year; and a minimum of two attacks per month. Concomitant tension-type headaches were permitted, provided their frequency did not exceed 15 attacks per month. Exclusion criteria were: presence of other forms of migraine; concomitant systemic or neurological diseases assessed by clinical examination; clinical evidence of vestibular diseases; overuse of analgesics — defined as intake in more than 4 days per week; pregnancy; breast feeding; and restrictions on MRI scans, such as claustrophobia or MR incompatible body implants. The study was approved by the ethics committee of the Hospital Universitário Clementino Fraga Filho, Universidade Federal do Rio de Janeiro (#004/06).

Computerized Static Stabilometry

Subjects were instructed to stand normally, barefoot, with arms relaxed alongside the trunk on a force platform device with a pressure sensitive sensor at each vertex. The apparatus was connected to a computerized recording system for data acquisition (LabView 5.0, National Instruments, Austin, TX) and analysis (MatLab 5.3, MathWorks). Signals were sampled (12-bit A/D conversion) and lowpass filtered with a second order Butterworth filter with a 5 Hz cut-off frequency. Centre of pressure (CP) displacements were used to evaluate the average amplitude of swaying both in the latero-lateral (x) and antero-posterior (y) axes, the total area of oscillation, the mean oscillation frequency in both axes and the total mean oscillation speed.

Two data acquisition blocks, each consisting of four successive one-minute periods, alternating closed eyes (CE) with open eyes (OE), were used to collect postural data. The two blocks were separated by a five-minute rest period, during which the subjects were allowed to sit and relax. The four acquisition steps within each block were separated one from another by 12-15 seconds, just sufficient for the examiner to ask the subjects to either close or open the eyes and activate the recording. The first acquisition was randomly chosen between the CE and OE situations and the first recordings with both OE and CE were discarded. Consequently, the final postural parameters were obtaining by averaging the 3 sets of data for both closed and open eyes. The following variables were considered for postural analysis: (a) for xSD and ySD, standard deviations of the amplitude of swaying in the x- and y-axis, respectively; (b) for VAvg, mean oscillation speed, all axes considered; (c) for Area95, area of sway after expurgation of the 5% outermost area; and (d) for FMx and FMy, mean oscillation frequency in the x- and y-axis, respectively. In addition, a Romberg Index (RI) was obtained from the division of the CE value by the OE value for each of the 6 stabilometric parameters.

Magnetic Resonance Imaging

Structural imaging: 3D reconstructions of the brain images were obtained using two high-resolution magnetization-prepared rapid acquisitions with gradient echoes (MP-RAGE) on a 3.0T scanner (Magneton Trio, Siemens, Erlangen, Germany) using an 8-channel head coil and the following parameters: 1.0x1.0x1.25mm; 128 slices; 256x256 matrix; echo time (TE)=3.44ms; repetition time (TR)=7.25ms; and flip angle=7°. The conventional MR imaging protocol also included axial FLAIR (2.0x2.0x5.0mm, TR=9950ms, TE=100ms, matrix 256x256 matrix, gap 35%) and T2-weighted images (2.0x2.0x3.0mm, TR=4410ms, TE=98ms, 320x320 matrix, gap 30%).

Diffusion Tensor Imaging: The DTI scans used a single-shot, twice-refocused echo planar sequence.17 Protocol parameters were: (a) forTR/TE, 9200/91ms, 2mm isotropic resolution, 64 slices, FOV 128x128mm, 1 average, 60 directions of diffusion encoding with b=700s/mm2, and 10 encodings with b=0s/mm2. Head motion was reduced by the use of padded clamps tightly attached to the head coil. Data were transferred to a workstation for off-line post-processing (Siemens Medical Solutions, DTI Task Card software, Massachusetts General Hospital).

Analyses of the FA maps were performed after correction for head orientation, eddy currents and susceptibilities. The same neuroradiologist, with 5 years of experience in this technique, defined nine regions of interest (ROIs) in the posterior fossa based on conventional MRI sequences and the FA maps: anterior middle cerebellar peduncle (0.4cm2, a-MCP); right (0.8cm2, r-MCP) and left (0.8cm2, l-MCP) middle cerebellar peduncles; right (0.2cm2, r-ICP) and left (0.2cm2, l-ICP) inferior cerebellar peduncles; right (0.3cm2, r-DN) and left (0.3cm2, l-DN) dentate nuclei; and right (3.0cm2, r-CWM) and left (3.0cm2, l- CWM) cerebellar white matter.

Statistics

The values were stored in a spreadsheet; Prism for Macintosh 4.0 (GraphPad Software, Inc.) and SPSS 12.0 (SPSS Inc. Chicago, Il, USA) for Windows were used for statistical analysis. The Student t-test and the Mann-Whitney test were used for group comparisons. FA values in all ROIs were correlated with the stabilometric parameters and the clinical data (linear regression), and statistical significance was considered for p-values < .05. Values are shown as mean ± standard deviation (min-max).

Results

Table 1 shows the demographic data. The MWA and MWoA patients were considered together in the FA analysis due to the small number of MR scans. The group of patients who underwent MR examinations was similar to the population as a whole, since no statistically significant differences in age, age at onset, timespan of disease, stabilometric and FA data were detected between this subgroup and the other patients. However, the 10 subjects with MRI examination reported more frequent headache attacks (p=0.0262).

Table 1. Demographic Data

Group		n	Age	A at O	Females	MTS	Freq
With MRI	MWA	3(6.25%)	*	**	3(100%)	***	****
	MHC	3(6.25%)	*****	-	3(100%)	-	-
	MWoA	7(14.5%)	28.8±6.6 (30, 16-40)	13.8±3.4 (14, 8-18)	7(100%)	15.0±6.3 (15, 2-22)	7.0±5.2 (4,2-16)
	MHC	7(14.5%)	28.2±7.5 (28, 12-42)	-	7(100%)	-	-
	Migraine	10(20.8%)	30.9±7.6 (30, 19-45)	14.4±5.2 (15, 6-24)	10(100%)	16.5±9.6 (15, 2-39)	6.5±4.5 (4,2-16)
	MHC	10(20.8%)	30.6±8.84 (29, 18-48)	-	10(100%)	-	-
	MWA	18(37.5%)	34.1±11.0 (31, 18-54)	17.6±9.3 (14, 7-35)	16(88.8%)	16.5±7.4 (17, 4-36)	7.8±10.1 (3. 0.2-30)
	MHC	18(37.5%)	34.6±11.7 (33, 19-55)	-	16(88.8%)	-	-
Without MRI	MWoA	20(41.6%)	35.0±11.8 (31, 21-62)	17.6±6.6 (17, 7-34)	16(90.0%)	17.4±13.0 (15, 1-40)	2.8±3.0 (2. 0.4-12)
	MHC	20(41.6%)	35.0±13.2 (30, 20-64)	-	18(90.0%)	-	-
	Migraine	38(79.1%)	34.5±11.3 (31, 18-62)	17.6±7.9 (16, 7-35)	34(89.4%)	16.9±10.6 (16, 1-40)	5.1±7.6 (2, 0.2-30)
	MHC	38(79.1%)	34.9±12.4 (32, 19-64)	-	34(89.4%)	-	-
Total Subjects	MWA	21(43.7%)	34.3±10.5 (32, 18-54)	17.3±9.1 (15, 6-35)	19(90.4%)	17.0±8.7 (17, 4-39)	7.4±9.4 (4, 0.2-30)
	MHC	21(43.7%)	34.8±11.3 (33, 19-55)	-	19(90.4%)	-	-
	MWoA	27(56.2%)	33.4±10.9 (30, 19-62)	16.6±6.1 (16, 7-34)	25(92.5%)	16.7±11.6 (15, 1-40)	3.9±4.0 (2, 0.4-16)
	MHC	27(56.2%)	33.3±12.3 (29, 18-64)	-	25(92.5%)	-	-
	Migraine	48(100%)	33.8±10.7 (31, 18-62)	16.9±7.5 (16, 6-35)	44(91.6%)	16.8±10.3 (15,.5, 1-40	5.4±7.1 (3, 0.2-30)
	MHC	48(100%)	34.0±11.8 (30, 18-64)	-		-	-

Legend: n: Number of subjects (% of total population); Age: Mean age in years; A at O: Age at onset in years; Females: Number of female subjects (% of females within the group). MTS: Migraine timespan in years. Freq: Estimated frequency of headache days per month. MRI: Magnetic resonance imaging. MWA: Migraine with aura. MWoA: Migraine without aura; migraine: Total migraine patients, with and without aura. MHC: Matched healthy controls.

* Ages of the three subjects were 27, 35 and 45 years. / ** Ages at onset of the three subjects were 17, 24 and 6 years.

*** Migraine timespan in the three subjects was 10, 11 and 39 years. / **** Frequencies in the three subjects were 4, 8 and 4. / ***** Ages of the three subjects were 27, 33 and 48 years. Age, age at onset, timespan of migraine and frequency of attacks are shown as mean±SD (median, minimum value – maximum value)

Stabilometry

All 6 basic CSS parameters showed no differences between controls and migraineurs as a single group, with both closed and open eyes. With regard to the RI, the only significantly different variable was xSD, which was higher in the migraine group. Comparing the migraine subtypes, FMx was higher in MWoA than MWA with both OE and CE. The RIs showed no differences for all stabilometric parameters comparing all migraine subjects or MWoA with MHC; as well as MWA with MWoA. However, the RI was significantly higher for the variable xSD in MWA as compared with MHC. The stabilometric data are shown in Table 2.

Table 2. Stabilometric data (total n=48)

Stabilometric parameter	Mean	SD	Mean	SD	p value
MWoA			MWA		
Open eyes					
xSD	0.33	0.12	0.34	0.21	0.8503
ySD	0.45	0.14	0.40	0.17	0.3261
VAvg	0.94	0.22	0.83	0.31	0.1652
Area95	2.89	1.83	3.15	4.32	0.7798
FMx	0.22	0.05	0.18	0.05	0.0078
FMy	0.19	0.06	0.19	0.04	0.9876
MWoA			MWA		
Closed eyes					
xSD	0.35	0.12	0.35	0.24	0.9513
ySD	0.45	0.13	0.42	0.21	0.4888
VAvg	1.03	0.27	0.93	0.37	0.2824
Area95	3.05	1.71	3.43	5.02	0.7164
FMx	0.23	0.19	0.19	0.05	0.0210
FMy	0.19	0.20	0.20	0.05	0.2649
All patients			MCH		
Romberg Index					
xSD	1.06	0.19	0.99	0.20	0.0494
ySD	1.03	0.20	1.05	0.22	0.7047
VAvg	1.12	0.19	1.11	0.18	0.8660
Area95	1.08	0.24	1.05	0.33	0.6829
FMx	1.11	0.81	1.15	0.27	0.442
FMy	1.12	0.27	1.14	0.33	0.8114

Table 2. Stabilometric data (total n=48) (cont.)

Stabilometric parameter	Mean	SD	Mean	SD	p value
	MWA		MHC		
Romberg Index					
xSD	0.93	1.13	0.36	0.14	0.0172
ySD	1.04	1.26	0.47	0.17	0.8460
VAvg	1.09	1.19	0.89	0.20	0.6749
Area95	0.94	1.16	3.36	2.20	0.3687
FMx	1.18	1.00	0.18	0.05	0.5638
FMy	1.12	1.19	0.17	0.06	0.7357

Legend: SD: Standard deviation; MWA: Migraine with aura (n=21); MWoA: Migraine without aura (n=27); MHC: Matched healthy controls; xSD and ySD: Standard deviations of sway amplitudes in the x- and y-axis, respectively; VAvg: Mean oscillation speed, all axes considered; Area95: Area of sway after expurgation of the 5% outermost area; FMx and FMy: Mean oscillation frequency in respectively x- and y-axis; Significant p-values are shown in bold.

MR and fraction anisotropy data

No lesion was noticed in conventional MR sequences in migraine and MHC. Table 3 shows the FA values in patients and controls. Patients had significantly lower FA values at the middle cerebellar peduncle and dentate nuclei bilaterally. On the contrary, the right cerebellar white matter showed significantly higher FA values in the group of patients. On the contralateral side with same ROI, FA was also higher in migraineurs, but the difference was not statistically significant (p=0,093).

Table 3. Fraction anisotropy (n=10)

ROI	Mean	SD	Mean	SD	p-value
	Migraine		MHC		
anterior-MCP	0.55	0.07	0.58	0.09	0.4309
right-MCP	0.60	0.08	0.69	0.06	**0.0109**
left-ICP	0.61	0.07	0.70	0.05	**0.0025**
right-ICP	0.57	0.05	0.61	0.04	0.1072
left-ICP	0.57	0.05	0.58	0.04	0.6876
right-DN	0.35	0.02	0.43	0.04	**0.0000**
left-DN	0.33	0.03	0.40	0.04	**0.0005**
right-CWM	0.29	0.01	0.26	0.03	**0.0028**
left-CWM	0.27	0.03	025	0.03	0.0930

Legend: ROI: Region of interest; SD: Standard deviation; MCP: Middle cerebellar peduncle; ICP: Inferior cerebellar peduncle; DN: Dentate nucleus; CWN: Cerebellar white matter; Significant p-values are shown in bold.

Clinical data correlations

Considering the group of migraine patients as a whole (n=48), the stabilometric parameters did not correlate with age in the OE situation. With CE, significant correlations were found between age and FMx (Pearson=0.30; p=0.033) and FMy (Pearson=0.38; p=0.0062). Both age and timespan of the disease correlated with the RI for VAvg (Pearson=0.33; p=0.02). Disease timespan also correlated positively with RI for FMx (Pearson=0.46; p<0.001). None of the parameters, with either CE or OE, correlated with age at onset and frequency of attacks. In MHC, age did not significantly correlate with any stabilometric parameter, with both OE and CE, and RIs.

The significant correlations between clinical data and stabilometry variables in the MR subpopulation (n=10) are shown in Table 4. No significant correlation in this subpopulation was found between any clinical parameter and FA.

Table 4. Clinical and stabilometric data correlations (n=10)

Variables correlated		Patients		Controls	
Clinical	**Stabilometric**	**Pearson**	**p-value**	**Pearson**	**P-value**
Age	VAvg OE	0.27	p=0.450	0.87	p=0.001
	VAvg CE	0.33	p=0.349	0.88	p=0.001
	FMx OE	-0.56	p=0.094	0.79	p=0.007
	FMy OE	0.61	p=0.060	0.90	p=0.001
	Area95 CE	0.51	p=0.131	0.67	p=0.034
	FMy CE	0.70	p=0.023	0.66	p=0.034
Timespan	FMy OE	0.08	p=0.010	-	-
	FMy CE	0.79	p=0.006	-	-
	Area95 CE	0.07	p=0.021	-	-
Frequency	ySD OE	-0.78	p=0.008	-	-
	FMx OE	0.07	p=0.039	-	-
	ySD CE	-0.67	p=0.027	-	-

Legend: xSD and ySD: Standard deviations of sway amplitudes in the x- and y-axis, respectively; VAvg: Mean oscillation speed, all axes considered; Area95: Area of sway after expurgation of the 5% outermost area.FMx and FMy: Mean oscillation frequency in the x- and y-axis, respectively.OE: Open eyes; CE: Closed eyes

Significant p-values shown in bold.

Stabilometry – fraction anisotropy correlations

Significant negative correlations were found between FA in different ROIs and various stabilometric parameters, particularly the r-DN with VAvg (OE and CE), l- CWM with xSD (OE and CE). In MHC, only two correlations were significant, in comparison with 8 in the migraine group (Table 5).

Table 5. Fraction anisotropy and stabilometric data correlations (n=10)

Variables correlated		Patients		Controls	
FA (ROIs)	**Stabilometric**	**Pearson**	**p-value**	**Pearson**	**p-value**
right-DN	VAvg OE	-0.68	p=0.031	-0.06	p=0.578
	VAvg CE	-0.71	p=0.019	-0.15	p=0.680
left-CWM	xSD OE	-0.73	p=0.016	-0.06	p=0.852
	Area95 OE	-0.65	p=0.042	-0.03	p=0.450
	xSD CE	-0.76	p=0.011	-0.36	p=0.299
	VAvg CE	-0.66	p=0.037	-0.09	p=0.805
anterior-MCP	VAvg OE	-0.196	p=0.587	-0.65	p=0.044
	ySD CE	0.735	p=0.015	-0.36	p=0.303
left-MCP	FMx CE	-0.522	p=0.122	-0.65	p=0.040
right-ICP	ySD CE	0.712	p=0.021	-0.01	p=0.987

Legend: FA: Fraction Anisotropy; ROI: Region of interest; xSD and ySD: Standard deviations of sway amplitudes in the x- and y-axis, respectively; VAvg: Mean oscillation speed, all axes considered; Area95: Area of sway after expurgation of the 5% outermost area; FMx and FMy: Mean oscillation frequency in the x- and y-axis, respectively. OE: Open eyes; CE: Closed eyes. Significant p-values shown in bold.

Discussion

Stabilometry objectively measures stance performance, and changes in fractional anisotropy indicate changes in brain tissue. Testing migraine patients interictally and controls with both methods may provide information on the possible existence of subclinical stance impairment in migraine, and indicate possible anatomical bases for such dysfunction. Stabilometric parameters did not differ between migraine and controls in a head-to-head comparison. However, patients with MWoA showed significantly higher frequency of lateral oscillations compared with those with MWA. Focusing on the RI results, MWA subjects as well as the entire migraine group showed higher indices for the lateral sway amplitude parameter, a finding not present in MWoA. Thus, MWA subjects were more dependent than MWoA patients on visual inputs to control the amplitude of lateral swaying but showed less frequency of swaying in this axis. No impairments were found concerning anteroposterior oscillations. This finding contrasts with Mauritz *et al.*, who suggested that cerebellar lesions, particularly at the anterior lobe, would often induce an increased frequency of swaying in the antero-posterior direction.[18]

In a previous study, no differences were found using stabilometry in migraine patients in any of the parameters for the experiments with eyes open, but balance in migraineurs was worse than in controls with eyes closed. The RI of all parameters were mostly higher in migraineurs in comparison to healthy volunteers.[19] The parameters used in this study, however, were distinct from ours, suggesting that differences may be related to methodological differences. The extension and speed of swaying have been found to be higher in the migraine posturograms,[13] suggesting that balance impairment may be present in this condition. These studies, however, did not detect a preferable axis oscillation dysfunction.

FA values were significantly lower at the middle peduncle and dentate nucleus in the patient group. In contrast, a clear tendency to relatively higher FA values was seen at the cerebellar white matter bilaterally. Lower FA values have been detected at the cerebellar peduncles in spinocerebellar degenerative diseases[20] and various ataxia syndromes, including spinocerebellar ataxia type 1 and multiple system atrophy.[21] Since no abnormality in T1, T2 and FLAIR sequences were detected in our patients, FA changes are probably unrelated to the posterior fossa subclinical infarct lesions previously described to be more frequent in migraine.[22] Lower FA was previously found in regions involved with migraine pathophysiology, such as the trigeminothalamic tract and the ventrolateral periaqueductal gray region.[23] The DN, the most lateral and phylogenetically recent of the cerebellar nuclei, receives afferents from the cerebellar cortex and various other structures, including the trigeminal sensory nuclei.[24] It is possible that the reduction of FA in this nucleus seen in migraine patients is related to the same mechanism that reduces FA at the periaqueductal gray and the trigeminothalamic tract. The cerebellum has been found to be involved with non-motor functions, such as cognition.[25, 26]

In addition, somatosensory stimuli have been shown to activate the cerebellum,[27] including the DN.[28] The DN is functionally divided into a dorsal, microgyric portion and a ventral, macrogyric portion. This nucleus has developed in great apes and humans to a greater extent, particularly due to an expansion in the relative size of the ventral half, an area related to non-motor functions.[26] DN sensory functions may be particularly impaired in migraineurs.

The middle peduncle is the main structure for projection to the cerebellar cortex from pontine nuclei, which receive input from the cerebral cortex, including visual areas.[25] In monkeys, there is a dense projection to the pons from the dorsal visual stream of extrastriate visual areas, where most of the neurons are motionsensitive; and there are few or no inputs from areas in the ventral stream of cortical visual areas, where cells are involved with higher visual processes, such as face recognition and form discrimination. Visual motion perception is known to be impaired in migraine interictally,[29] an abnormality not caused by lack of attention.[30] The influence of vision in reducing the amplitude of lateral sway was detected by a significantly higher RI in MWA. In addition, the frequency of lateral oscillations was comparatively shorter in MWA as compared with MWoA, but not in relation to controls. Dysfunctions in visual perception, possibly more impactful in MWA concerning lateral sway during stance, may explain the present findings. Speculatively, cortical-ponto-cerebellar dysfunctional fibers originating in the migraineur's visual areas could contribute to the FA changes at the middle peduncle.

FA reduction does not necessarily imply a tissue lesion. From a functional perspective, an increase in axonal diameter secondary to over-functioning, as previously speculated, could lead to reduced FA.[23] On the contrary, if this assumption is correct, functional changes in an opposite direction could explain FA increases with decreases in axonal diameter, such as found at the cerebellar white matter in migraineurs. However, the mechanisms that govern such reactions and their reasons remain obscure. Taken together, the CSS and the FA data seem to indicate dysfunctions of output/input to the cerebellum involving visual and trigeminal pathways, rather than direct cerebellar lesions. In the right DN, but not the left, a negative correlation was found between the mean oscillation speed and FA in migraineurs. This suggests that increased anatomical dysfunction is associated with higher oscillation speeds. Asymmetrical activation of the DN, greater at the right side, was noted with both bilateral28 and left27 somatosensory stimulation. Overstimulation of the sensory pathways involved in migraine pathophysiology could therefore lead to asymmetrical dysfunction of the DN, causing a significant correlation with FA-sway speed only on the right side. In migraineurs, negative cor-

relations were also found between the left cerebellar white matter and the amplitude of lateral sway as well as the area of sway (situation OE) and the oscillation speed (situation CE). The reasons for such findings are unknown.

Clinical parameters were correlated with both stabilometric (total patients, n=48 and the MR subgroup, n=10) and FA (n=10) data. Findings seemed scattered and puzzling. The MR subgroup showed significant correlations concerning frequency of attacks for three stabilometric variables (negative for ySD with OE and CE; positive for FMx), but frequency correlations were not significant in tests of the population as a whole. This may be explained by the fact that, with regard to frequency, the MR subgroup was by chance not representative of the whole group because of more frequent headache attacks. Both the total migraineur and the MR subgroup showed positive correlations between age and stabilometric parameters, a finding not present in MHC, when the total patient group is considered but present for some parameters in the MR subgroup. These findings are inconsistent and must be regarded as the result of chance after multiple comparisons. The timespan of the disease showed positive correlations with 5 parameters in the MR subgroup, but only 2 when considering the whole group of migraineurs; variables distinct from the 5 were found to correlate with the timespan of the disease in the smaller group. Until proven otherwise, the correlation of clinical parameters with stabilometric data should not be considered as relevant. Similarly, no correlation was found between FA and clinical data. FA was shown to vary with age.[31] In our population, the lack of correlation between FA and age may be explained by the relatively small population sample and by the fact that migraine tends to be concentrated in young patients.[32]

Conclusion

In conclusion, our data suggest that migraine may interfere with the anatomy of the cerebellum, especially the DN and middle cerebellar peduncles, as estimated by diffusion tensor MR imaging. DN abnormalities with tendency to be mild dysfunctional changes may result in interictal subclinical imbalance characterized by impairment of oscillation in the lateral axis, especially in MWA patients, who may be more dependent on visual input to control lateral sway than MWoA subjects.

Acknowlegdments

The authors are indebted to Professor Ronir R. Luiz, for his assistance with statistical analysis, Jose M Oliveira for his assistance with stabilometry and Emerson Gasparetto for his assistance with MRI.

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Update on Aztreonam and Prospects for the Development of New Drugs

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Abstract

Introduction: Monobactams belong to the beta-lactam group and are drugs indicated for the treatment of infections caused by aerobic Gram-negative bacteria. Aztreonam is currently the only available representative of the group and, due to its clinical safety profile, efforts have been directed towards the development of new antibacterials in this class. Methodology: This is a narrative literature review that examines articles, books, and documents from various sources in Portuguese, English, and Spanish. The analysis focuses on relevant data regarding aztreonam and related compounds, addressing the main aspects of aztreonam – brief history, chemical structure, mechanism of action, pharmacokinetics, pharmacodynamics, spectrum of action, resistance mechanisms, drug interactions, clinical indications, dosage, use in special situations, and adverse effects – as well as presenting the potential of the aztreonam-avibactam combination and the development of new monobactams. Results and Discussion: Aztreonam has a unique chemical structure, containing a monocyclic beta-lactam ring. Its bactericidal action occurs through the inhibition of penicillin-binding protein-3 (PBP-3), affecting the cell wall synthesis of Gram-negative bacteria. In general, the drug is well-tolerated and causes mild adverse effects. Combinations like aztreonam-avibactam are promising for treating infections caused by multi-drug-resistant bacteria. Studies highlight the need for new compounds, such as LYS228 and BAL30072, which expand the spectrum of action and overcome resistance mechanisms. Conclusion: Aztreonam remains relevant in antimicrobial therapy against Gram-negative infections. The development of new monobactams is a strategy to address increasing bacterial resistance and improve the treatment of complex infections.

Keywords: aztreonam, beta-lactams, monobactams.

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BJHBS, Rio de Janeiro, 2025;24(1):38-48
DOI: 10.12957/bjhbs.2024.85386
Received on 30/11/2024. Approved on 18/03/2025.

Introduction

Beta-lactams are antimicrobial drugs that have the homonymous ring – which is responsible for antibacterial action – in their structure and whose scope includes four main groups of drugs: penicillin, cephalosporins, carbapenem and monobactams.[1] This latter group was described in 1975, in Japan, when researchers detected the production of beta-lactam antibiotics formed by a monocyclic structure – the monobactams –, by bacteria of the species *Nocardia uniformis*.[2]

Further research led to the production of aztreonam, the only drug available in this class, which stands out mainly for its stability against most beta-lactamases.[3] It is a bactericidal antibiotic that interferes with cell wall synthesis and causes bacterial lysis through interaction with penicillin-binding proteins (PBP) of sensitive microorganisms, inducing the formation of long bacterial filaments.[4,5] For some authors, the mechanism of action of aztreonam is similar to that of aminoglycosides, that is, it diffuses through the protein pores in the outer membrane, reaches the periplasmic space and penetrates the inner membrane by means of electron transport. Since this transport depends on energy, anaerobic environments do not permit the penetration of the drug into the cell, which reduces or completely stops its microbicidal action.[6,7]

Moreover, the low binding of the drug to PBP3 in gram-positive pathogens renders aztreonam ineffective against these etiological agents. Consequently, its spectrum of activity is limited to aerobic gram-negative bacteria, so other antimicrobials must be combined to broaden the coverage to include gram-positive and anaerobic bacteria.[7–9]

The medical importance of aztreonam lies in its in vitro activity against most strains of Enterobacteriaceae, even those that are multidrug-resistant, a context that represents a significant public health problem today.[10,11] In addition, aztreonam is able to act on Gram-negative non-sugar fermenters that cause healthcare-related infections – such as *Pseudomonas aeruginosa* – without the intrinsic nephrotoxicity of aminoglycosides.[1,8] In fact, the in-depth and constant study of aztreonam is of great importance, since it is effective against these pathogens (*Enterobacteriaceae* and *P. aeuruginosa*).[12]

Based on these considerations, the objectives of this article are [1] to review the most important features of aztreonam – historical, chemical, pharmacological (mechanism of action, pharmacokinetics, pharmacodynamics, spectrum of action, mechanisms of resistance and drug interactions) and therapeutic (clinical indications, dosage, use in special situations and adverse effects) aspects – and [2] discuss the prospects for the use of the aztreonam-avibactam association and for the development of new drugs of the monobactam class.

Methodology

This paper is a narrative review that aims to describe a specific theme or point of view in a theoretical and contextual way[13] that is important for professional training – and also for lifelong learning –, since it provides readers with the ability to update knowledge in a shorter period of time, although it doesn't provide the methodological data that allows the reproducibility of the search for bibliographic references.[14] Therefore, the design of this study is distinct from a systematic review, which, despite being better structured, has the limitation of answering a specific question through a careful evaluation of studies that provide data – which are collected and analyzed –, in order to support the approach to the proposed question.[13] It should also be highlighted that the literature review is the first step in the synthesis of scientific knowledge, allowing gaps to be filled and opportunities for new research to be seized.[13,15]

Based on these concise notes, references were gathered from articles, books and published documents (produced by government agencies, scientific institutions and specialized bodies), in Portuguese, English and Spanish. The papers selected were read in full to summarize the relevant data, which allowed – through a critical analysis by the authors – the elaboration of results and discussion of the following topics: brief history; chemical structure; mechanism and spectrum of action; pharmacokinetics and pharmacodynamics; resistance mechanisms; drug interactions and adverse effects; clinical indications and dosage; use in special situations; and new monobactams.

Results and Discussion

Brief History

Beta-lactams represent a class of antibiotics of great clinical importance worldwide.[16] The first drug of this class was discovered in 1928 by Alexander Fleming, who noted that a culture of *Staphylococcus aureus* contaminated by a fungus, currently known as *Penicillium chrysogenum*, had its growth inhibited by the action of penicillin.[17]

The description of penicillin G was a milestone for the development of antimicrobial therapy. Since then, other beta-lactams have been made available for the treatment of different infectious conditions, thus revolutionizing the care of people with bacterial infections.[16,18] After the identification of penicillins and cephaloporins, other drugs emerged to increase the spectrum of action and potency of preexisting agents, including monobactams.[19]

The description of monobactams originates from the work of Aoki and collaborators, who identified a monocyclic beta-lactam antimicrobial—initially named nocardicin A—from the fermentation broth of a strain of *Nocardia uniformis subsp. tsuyamanensis*.[20] This isolated compound was the first monobactam discovered, although its antimicrobial potency was too low for clinical application.[4] Subsequent research led, about five years later, to the development of aztreonam—the first, and so far the only, monobactam approved for clinical use. Aztreonam is a synthetic drug developed in 1981, based on structural modifications of the natural monobactam produced by the Gram-negative bacterium *Chromobacterium violaceum*.[4,21]

Chemical Structure

The presence of a beta-lactam ring (β-lactam or beta-lactam), formed by four atoms (ring of four members) is the main characteristic of the chemical structure of aztreonam.[22,23] Lactams are cyclic amides, whose rings are characterized by nitrogen as a heteroatom directly linked to a carbonyl carbon. In the structure of a lactam, the first carbon atom attached directly to the carbonyl is designated alpha (α) and the next carbon atom is designated beta (β). Thus, the term β-lactam indicates that carbon β (in relation to carbonyl) is also bound to nitrogen, characterizing a ring formed by four atoms.[24] The β-lactam ring is present in the chemical structure of a series of antibiotic families called β-lactams antibiotics – including penicillins, cephalosporins, carbapenems and monobactams – and constitutes a fundamental structural portion of the antibacterial activity of all these classes.[25,26] Chemically, the simplest β-lactam, whose atoms that constitute the cycle are not bound to any substituents, is called azetidin-2-one (or 2-oxazetidine). Thus, β-lactams are structurally derivatives of azetidin-2-ones.[27] Aztreonam, whose chemical name is acid 2-[[[1-(2-amino-4-thiazolil)-2-[(2-methyl-4-oxo-1-sulfo-3-azetidinil)amino]-2-oxoetilideno] amino]oxy]-2-methyl-[2S-[2α,3β(Z)]], is a synthetic antibiotic of the monobactam class. Unlike penicillins, cephalosporins and carbapenems, for example, the chemical structures of monobact-

ams do not contain a second ring fused to the β-lactam ring. The name monobactam is thus a reference to the monocyclic structure of this class of β-lactams,[22] as shown in the figure below.

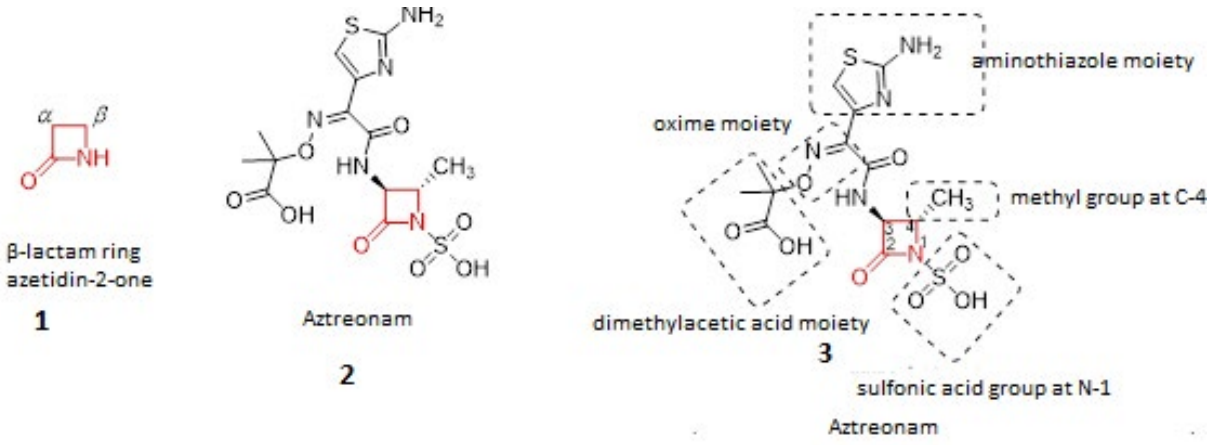

Figure 1. Chemical structure of the β-lactam ring and aztreonam
Source: The authors (2025).

All naturally occurring monobactams have a nitrogen-bound sulfonic acid (-SO3H) group (N-1) in the β-lactam ring, characterizing a structural portion of azetidin-2-one-1-sulfonic acid, along with a variety of substituents linked to carbon 3 of this ring. From the chemical structure of these natural monobactams, Structure-Activity Relationship (SAR) studies led to the development of the chemical structure of aztreonam. In this, the sulfonic acid group linked to N-1 promotes activation of the β-lactam ring. The substituting methyl group in carbon C-4 (β position) increases the stability of the β-lactam ring against the action of β-lactamases, promoting increased antibacterial activity. The aminotiazole oxime portion in the lateral acyl chain linked to C-3 of the β-lactam ring is responsible for potent activity against aerobic Gram-negative bacteria. The two methyl groups and the carboxylic acid group, which constitute the dimethyl acetic acid portion, linked to the oxime group, result in an optimization of the activity against a broad spectrum of Gram-negative bacteria.[22,28,29]

Mechanism and Spectrum of Action

Aztreonam is a bactericidal antibiotic whose mechanism of action is related to the presence of the beta-lactam ring, and it does not induce the production of beta-lactamases. This mechanism involves the interruption of bacterial cell wall synthesis and bacterial death through binding to penicillin-binding protein 3 (PBP-3). The polymerization process required for the formation of peptidoglycan—a key structural component of the bacterial cell wall—depends on the activity of various enzymes, including transglycosylases, transpeptidases, carboxypeptidases, and endopeptidases. PBPs, located on the outer surface of the plasma membrane, exhibit catalytic activity corresponding to these enzymes.[30] Their inhibition by antimicrobials—such as aztreonam—disrupts the synthesis and incorporation of peptidoglycan into the bacterial cell wall.[31] Since autolysins—autolytic enzymes responsible for breaking down the aged peptidoglycan to enable the construction of new cell wall layers—remain active, the ultimate consequence is the formation of defective walls, or even the failure to form them. This results in osmotic lysis of the bacterial cell, caused by the influx of water from the hypotonic external environment into the cell's interior.

The high selectivity of aztreonam for Gram-negative bacteria is due to its strong affinity for the PBPs of these organisms. Its minimal or absent in vitro activity against Gram-positive and an-

aerobic microorganisms results from the weak binding of the drug to the PBPs of these bacteria. However, compared to other monobactam antibiotics, the bactericidal and bacteriostatic concentrations (inhibitory concentrations) of aztreonam have been observed to be very similar.[4]

Aztreonam has specificity against aerobic Gram-negative bacteria. It has high resistance to inactivation by beta-lactamases, both those of plasmid and chromosomal origins (all class B and most classes A and D), which makes this antibiotic efficient even against cephalosporin-resistant microorganisms. Most *Enterobacteriaceae* of community origin are normally sensitive to aztreonam, even at low concentrations (less than 1µg/ml).[32] Currently, Gram-negative bacilli of nosocomial origin may present resistance to aztreonam. It has exclusive activity against aerobic Gram-negative bacteria, except the strains producing beta-lactamases KPC, extended spectrum (ESBLs) and ampicilinase class C (AmpC).[6,21,33] However, most strains of *Enterobacteriaceae*, *Haemophilus influenzae*, *Moraxella catarrhalis*, *Neisseria gonorrhoeae*, *Neisseria meningitidis*, *Aeromonas spp.* and *Pasteurella multocida* show sensitivity at low concentrations of this drug. *P. aeruginosa* generally requires high inhibitory concentrations and about a third of the strains of this bacterium are currently resistant to aztreonam. Most strains of *Burkholderia cepacia*, *Stenotrophomonas maltophilia*, *Citrobacter freundii*, *Enterobacter aerogenes* and *Campylobacter jejuni* are resistant to the drug, as are *Legionella pneumophilia*, *Mycoplasma spp.*, *Acinetobacter spp.* and *Chlamydia spp*.[8,21,32–34]

Pharmacokinetics and Pharmacodynamics

Aztreonam may be administered intravenously (IV), intramuscularly (IM), inhaled, and intraperitoneally. Bioavailability after oral administration is <1%. Its mean serum elimination half-life is two hours in patients with normal liver and kidney functions. Aztreonam has 56% serum protein binding and good tissue distribution, penetrating most tissues and body fluids, including bones, prostate, bronchial secretion and liquor. About two-thirds of the drug is eliminated unchanged in the urine, with glomerular filtration and tubular secretion playing approximately equal roles. Serum clearance of aztreonam after a dose of 1g IV was linearly related to clearance of urinary creatinine.[21,32,34,35]

Like other beta-lactams, aztreonam is characterized by a time-dependent bactericidal effect. Data obtained in animal models suggest that the efficacy of aztreonam is related to a time interval greater than the minimum inhibitory concentration (MIC) (t>MIC) of 50% to 60% of the dose interval. Nevertheless, limited pharmacodynamic data suggest that the efficacy of aztreonam in humans is more closely related to the ratio of the area below the time-concentration curve (AUC) over the MIC (AUC/MIC).[35]

Resistance Mechanisms

Resistance to aztreonam is usually due to the action of beta-lactamase enzymes, which hydrolyze the beta-lactam ring. Two molecular mechanisms employed by beta-lactamases may occur: action of the nucleophile serine (class A and D beta-lactamases) or production of chromosomal or plasmid AmpC. Class B beta-lactamases (metalobeta-lactamases) are unable to hydrolyze aztreonam.[36–38]

Other mechanisms of resistance to aztreonam were described: increased expression of efflux pumps (mainly MexAB-OprM); and a deficiency of porin OmpK35 that causes a decrease in membrane permeability.[33,37]

Drug interactions and Adverse Effects

Aztreonam should not be administered concomitantly with sodium naphthyline, cefradine and metronidazol[39] (AZANEM® patient package insert). For the verification of other possible drug interactions, sites that are specialized in this subject (e.g. Drug Interactions Checker – https://www.drugs.com/drug_interactions.html and Drug Interactions – www.greghi.com.br/index.php) should be consulted.

When administered IV, aztreonam is a well-tolerated drug; however, about 2% of patients may develop phlebitis, usually after a week of use. When administered IM, the most commonly described adverse effects are pain and edema at the site of application. Rash was reported in 1% of the treated patients. Nausea, vomiting, diarrhea, dysgeusia, thrombocytopenia, leukopenia, elevated serum aminotransferases and changes in alkaline phosphatase and prothrombin activity are rarely reported adverse events.[4]

The antimicrobial should be used with caution in patients with a history of hypersensitivity to penicillins and cephalosporins, although cross-sensitivity is very unusual with the use of aztreonam.[4,40,41] However, in patients with a history of hypersensitivity to ceftazidime, administration of aztreonam should be even more cautious, since both antibiotics share an identical side chain. Although it can cause hepatotoxicity in children and infants, its use is generally well tolerated in this age group.[6]

Clinical Indications and Dosage

Aztreonam is indicated in the treatment of infections caused by aerobic Gram-negative bacilli, especially those acquired in the community, and the need for association with other antimicrobials for coverage of Gram-positive and anaerobic bacteria should be considered. When used to treat urinary tract, gynecological, pulmonary and osteoarticular infections, good cure rates are obtained.[4] In hospital infections, aztreonam is indicated in the treatment of pneumonia and urinary infections caused by sensitive bacteria.[6,42] In short, aztreonam represents an option in the treatment of infections caused by sensitive Gram-negative bacilli, without the potential for nephrous/ototoxicity that is inherent in the use of aminoglycosides.[1,32]

When combined with clindamycin, chloramphenicol and metronidazole, aztreonam can be used to treat intra-abdominal infections – such as peritonitis, appendicitis and liver, intra-abdominal, subphrenic and parietal abscesses[4,43] – in view of the action of these drugs in anaerobes. Moreover, it represents an alternative in the treatment of meningoencephalitis caused by *H. influenzae*, *N. meningitidis* and *Enterobacteriaceae*, especially in patients with a history of allergy to penicillins and cephalosporins.[1,33]

Aztreonam is generally used in the treatment of infections by *P. aeruginosas* or *Enterobacteriaceae*;[44] however, when compared to other beta-lactams, its use has been associated with an increase in lethality to patients with septic shock and the need for association with other antibiotics in infections by *Pseudomonas spp*.[45]

Although aztreonam is an option in the treatment of patients allergic to penicillin, more detailed research is needed regarding this hypersensitivity; if an IgE-mediated allergic reaction prior to penicillin is confirmed by a penicillin skin test or duly documented history, the probability of real cross-reactivity should be borne in mind, especially in patients with cystic fibrosis.[7]

A small clinical trial demonstrated that aztreonam 2g, via IM, is likely effective in the treatment of uncomplicated but ineffective gonococcal urethritis in the eradication of *N. gonorrhoeae* from the oropharynx. This treatment may be useful for rectal infections, but further research is needed before aztreonam can be recommended in the treatment of gonorrhea in this topography.[46] Although clinical data from more than three decades ago were used, a recent meta-analysis concluded that aztreonam could be an effective alternative in the treatment of urogenital gonorrhea, particularly among those with beta-lactam allergies. However, more data on the efficacy of treatment for pharyngeal and rectal infections, as well as information on the MIC distribution of contemporary isolates, are required before recommending this antimicrobial.[47]

Aztreonam (formulation containing lysine) can be administered by inhalation (nebulization) in the treatment of chronic infections by *P. aeruginosa* in patients with cystic fibrosis, at a dose of 75mg, three times a day.[48]

In patients allergic to beta-lactams, aztreonam associated with vancomycin was effective in the treatment of febrile[9] neutropenia, despite not being considered the antimicrobial scheme of choice for this clinical indication.[33]

Aztreonam (2 grams IV every 8h, infused in 3h) administered concomitantly with ceftazidime-avibactam (2.5 grams IV every 8h, infused in three hours) is an option in the treatment of infections caused by carbapenem resistant enterobacteria (CRE) producers of metallo-β-lactamases and in the rescue treatment of infections caused by *S. maltophilia*.[49–52]

In the treatment of infections of moderate severity, the recommended dose of aztreonam is 1 to 2 grams, by IV or IM, every 8 or 12 hours. In more severe infections, the suggested dose is 2 grams, IV, every 6 or 8h (maximum dose of 8 grams/day). In children, the suggested dose is 30mg/kg, IV, every 6 to 8h (maximum dose of 8 grams/day). In preterm infants (<2kg), the recommended dose is 30mg/kg, IV, every 12h.[32,33]

Currently, given the increasing number of hospital infections caused by multidrug-resistant bacteria, the use of aztreonam should be rationalized to preserve its efficacy. Aztreonam-avibactam (ATM-AVI) is a promising new therapeutic option to combat multidrug-resistant Gram-negative bacteria, including those that produce metallo-β-lactamases. Therefore, avoidance of the use of aztreonam in infections caused by multidrug-resistant bacteria is recommended, especially in community-acquired infections, in order to minimize selective pressure and reduce the risk of resistance. Furthermore, in critically ill patients with severe hospital infections, the therapeutic decision should be guided by in vitro sensitivity testing and rigorous monitoring of the clinical response.[52, 68]

Use in Special Situations: Pregnant women, lactating women, liver failure and kidney failure

Aztreonam proved to be a highly safe and effective drug (risk category A), according to pharmacokinetic and clinical studies carried out in the perinatal period (period between 22 completed weeks of gestation and 7 days after birth), in addition to having no side effects and no abnormal laboratory records.[39,53] Aztreonam is found in small amounts in breast milk and is considered an acceptable drug for use in lactating women.[33]

The half-life of aztreonam is slightly increased in hepatopaths, but there is no need to adjust doses in patients with chronic hepatopathy if there is no concomitant renal failure.[21,35]

Aztreonam is depurated through continuous venous hemofiltration, hemodialysis and peritoneal dialysis.[21] For patients with creatinine clearance between 10 and 30ml/min, the initial dose is recommended at the usual intervals on the first day of treatment, followed by half the initial dose at the usual intervals on subsequent days. For patients with creatinine clearance less than 10ml/min, the initial dose is recommended at the usual intervals on the first day of treatment, followed by a quarter of the initial dose at the usual intervals on subsequent days. A supplementary dose of 0.5 grams should be administered after each hemodialysis session.[32,44]

Aztreonam-Avibactam and new monobactams

Since their description, beta-lactams have been one of the most important and used antibiotic classes in the world. However, their overuse has led to the appearance of multidrug-resistant bacteria, many of which are no longer susceptible to existing drugs. This phenomenon reinforces the need for compliance with the principles of rational use of antimicrobials.[54] Therefore, strategies must be devised to overcome bacterial resistance,[55] the most efficient of which is the modification of the chemical structure of drugs already in use, promoting a change in their biological activity.[27]

Seeking the development of new monobactam agents with greater stability against the action of beta-lactamases and better activity against Gram-negative bacteria, Thu and collaborators (2021) synthesized six new compounds based on the structure of aztreonam. The azetidin-2-one ring with sulfonic acid group in N-1, which constitutes the central portion of the aztreonam molecule, was conserved in all of the synthesized compounds. The modifications made to the model compound consisted of: (i) introduction of a second methyl group in C-4 of the beta-lactam ring to increase the steric impediment in this position and, consequently, increase stability against beta-lactamases; (ii) replacement of the apolar geminal dimethyl group attached to the alpha carbon and the carboxylic acid group with a phenyloxy group aims to increase the polarity of this region of the molecule and enhance its interaction with bacterial macromolecules.; and (iii) introduction of a piperidine ring linked to the phenyloxy group through a urea portion, a structure that could act as siderophore and favor the entry of the compound into Gram-negative bacteria via bacterial iron absorption systems. The compounds differed from each other by the stereochemistry of a stereogenic center in the piperidine ring and the position in the nitrogen atom in this same ring, and also by the configuration (Z or E) of the double bond of the oxoimine portion. Two of these compounds showed higher potency against almost all bacteria tested compared to aztreonam.[27]

Studies have also shown that investigating the activity of aztreonam against beta-lactamases is important for supporting the discovery of new enzyme inhibitors. The selectivity of these enzymes increases with more hydrophobic compounds, and the addition of a chlorine atom has been shown to enhance the antibacterial activity of the synthesized drugs.[56]

Aztreonam associated with avibactam (non-beta-lactamase inhibitor) has in vitro activity against class A beta-lactamase-producing enterobacteria (including ESBLs and KPC), class B (metalobeta-lactamases), AmpC, some of class D (OXA-48) and *S. maltophilia*.[50,57–60] Nevertheless, such a combination is not effective in vitro against *Acinetobacter spp.* and some strains of *P. aeruginosa*, which could be explained by the presence of other resistance mechanisms, in addition to the production of beta-lactamases.[61,62] Moreover, aztreonam-avibactam is more susceptible to the "inoculum effect" than the combination of ceftazidime with avibactam.[63] Currently, aztreonam-avibactam is under evaluation in phase III clinical trials and may be useful in treating

patients allergic to penicillin, sparing the use of carbapenems. In addition, its use can be considered in patients at high risk of developing pseudomembranous colitis, since aztreonam hardly alters the intestinal anaerobic microbiome, due to the lack of activity of the drug against such bacteria.[37]

Two new monobactams incorporated a siderophore substructure to facilitate their absorption. The siderophore dihydropyridone substituent of BAL30072 confers a potent inhibitory activity against *Acinetobacter spp.*, *Burkholderia spp.* and *Pseudomonas spp.* as well as many species of Enterobacteriaceae. BAL30072 is highly resistant to hydrolysis by metalobeta-lactamases and is an AmpC inhibitor. BAL30376 is a triple combination antibacterial, consisting of a monobactam siderophore (analogous to aztreonam, with a dihydropyridone iron-chelating group), a class C beta-lactamase inhibitor (BAL29880, a structurally unique monobactam) and clavulanic acid. It has *in vitro* activity against the enzymes AmpC and ESBLs, and also against strains of *P. aeruginosa* resistant to carbapenemas, among other Gram-negative MDR.[64]

LYS228 is a new monobactam that has in vitro activity against metallobete-lactamases (NDM). However, due to changes in its chemical structure, it is also active against beta-lactamases of group A (KPC) and D, through its binding to PBP3.[3,65] Pharmacokinetic studies have shown that such an antimicrobial is safe and well tolerated.[65] It is currently in stage of phase II clinical trials (treatment of complicated urinary tract and intra-abdominal infections).[3]

Another recently developed monobactam, AIC499, has broad antimicrobial activity in vitro against strains of Gram-negative bacteria (*E. coli* and *P. aeruginosa*) and greater resistance to most beta-lactamases.[64,66] *In vitro* studies and the use of animal models have shown that a new antimicrobial, monosulfactam 0073, is a promising candidate for use as a single antibiotic against serine beta-lactamases and metalobete-lactamases produced by Gram-negative MDR.[67]

Conclusions

The scope of this article was to review of the main aspects of monobactams – which concentrated on the chemical, pharmacological and therapeutic aspects of aztreonam – and provide an update on the aztreonam-avibactam association and the current state of development of new monobactams.

Scientific efforts, in the form of new studies aimed at this group of drugs – whose action on aerobic Gram-negative bacteria is an important issue today –, may contribute to the development of innovative perspectives in terms of antimicrobial therapy, thus diversifying the possibilities of response to the increasing bacterial resistance to drugs, while especially expanding the potential for health care of people victimized by different infectious conditions.

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Trends and Insights into Glymphatic System Research and Alzheimer's Disease: A Bibliometric Analysis from 2014 to 2023

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Abstract

Introduction: Over the past decade, research on the glymphatic system has garnered increasing attention within the scientific community. This system, initially explored through animal models and magnetic resonance imaging tracer-based studies, holds potential implications for understanding neurodegenerative diseases, notably Alzheimer's disease. However, a bibliometric analysis of research related to the glymphatic system and Alzheimer's disease is lacking. Objective: Our study aimed to conduct a comprehensive analysis of the existing literature on glymphatic system research, with a particular focus on its intersection with studies of the pathology of Alzheimer's disease. By synthesizing available data, we sought to identify trends, gaps and potential avenues for future research and collaboration in this evolving field. Methods and resources: We utilized statistical techniques to analyze data obtained from the Web of Science Core Collection database. Our methodology encompassed various aspects, including frequency of publication, geographical distribution, citation networks, and thematic analysis of authors' keywords. Results and discussion: Our analysis covered a wide range of publications spanning the last decade and revealed a gradual increase in research output over time. Notably, we observed a significant level of international collaboration, underscoring the global nature of scientific inquiry in this domain. However, disparities in research capacity and collaboration were apparent, particularly among regions with limited resources. Conclusion: Our findings highlight the importance of continued interdisciplinary collaboration and exploration to advance our understanding the function of the glymphatic system and its relevance to neurodegenerative disorders. Addressing disparities in research capacity and fostering global collaboration are essential steps toward developing effective interventions for Alzheimer's disease and related conditions.

Keywords: Glymphatic System, Alzheimer's Disease, Bibliometric Analysis, Neuroscience, Neuroimaging.

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BJHBS, Rio de Janeiro, 2025;24(1):49-61
DOI: 10.12957/bjhbs.2024.85281
Received on 29/12/2024. Approved on 10/01/2025.

Introduction

In recent years, the glymphatic system (GS) hypothesis, which arose from a series of experiments conducted on animal models,[1–5] has gained significant momentum. Furthermore, magnetic resonance imaging (MRI) tracer-based studies have suggested a human equivalent of the GS in the brain, thus corroborating earlier findings in rodents obtained through the intrathecal administration of gadolinium-based contrast agents6. Despite ongoing debates regarding the validity of this hypothesis,[7,8] numerous studies investigating the waste clearance function of the central nervous system (CNS), including the GS, have been conducted.[9,10]

Recent literature suggests that the GS has significant implications for neurodegenerative diseases such as Alzheimer's disease (AD), in which compromised waste clearance mechanisms play a role in disease progression.[11,12] According to the classic amyloid cascade hypothesis,[13] the accumulation of β-amyloid peptide (Aβ) is an early event in AD pathogenesis. The progression of the disease, including the formation of neurofibrillary tangles containing tau protein, is a consequence of an imbalance between Aβ production and clearance.[14]

Given the growing interest within the neuroscientific community, we conducted a bibliometric analysis to assess the research landscape. With mounting evidence supporting the existence of this system in both animal[15] and human brains[16,17], our aim was to explore the extent to which research on this topic intersects with studies of AD pathology. Moreover, our work could catalyze further exploration and foster collaboration to advance our understanding of neurodegenerative diseases and develop more effective therapeutic interventions.

Previous studies have primarily focused on bibliometric analysis of the GS,[18] while our study explores the correlation between GS research and AD pathology. This distinction is crucial, because it provides novel insights into the intersection of these fields and offers a unique perspective on the relationship between GS function and neurodegenerative diseases.

Methods

Methodological Design and Data Retrieval

Our data retrieval process, conducted in May 2024, involved querying the Web of Science Core Collection (WoSCC) using pre-selected Mesh terms to ensure a comprehensive coverage of the relevant literature. The search query included terms such as "Glymphatic System," "Glymphatic Pathway," "Glymphatic Pathways," "Glymphatic Clearance System," "Meningeal Lymphatic Vessels," "Meningeal Lymphatic Vessel," "Brain Perivascular Spaces," "Virchow-Robin Spaces," and "Virchow-Robin Space", combined with terms related to AD such as "Alzheimer's Diseases," "Alzheimer Diseases," "Alzheimer Dementia," "Alzheimer Type Dementia," "Focal Onset Alzheimer's Disease," "Early Onset Alzheimer Disease," and "Late Onset Alzheimer Disease" (Figure 1). To refine the dataset, we included original journal articles, reviews, and early access publications, focusing on studies published between 2014 and 2023 to capture recent advancements. We excluded articles that did not meet these criteria or were not written in English, as well as conference abstracts, editorials, letters and other non-peer-reviewed materials, thereby ensuring the integrity and scholarly rigor of our analysis. See Supplementary Material for the detailed search strategy.

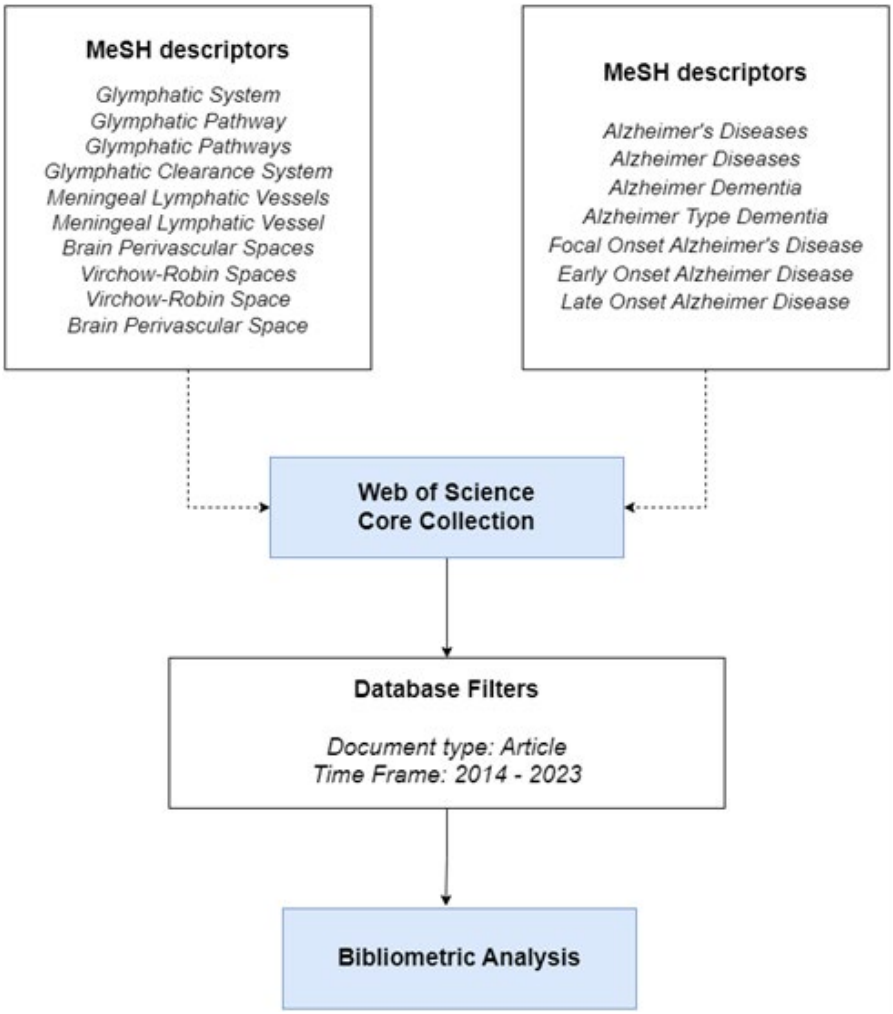

Figure 1. Methodological sequence flowchart.
Source: The authors (2024).

After data extraction, we implemented a filtration process to refine the dataset. This process included the inclusion of specific document types, such as original journal articles, reviews and early access publications, to ensure the integrity and relevance of the analysis. Temporal constraints were applied by focusing on publications from 2014 to 2023, thus capturing recent advancements and trends in the field.

We conducted a multifaceted analysis utilizing algorithms and statistical techniques to explore various dimensions of the dataset. These dimensions included frequency of publication, geographical distribution, institutional affiliations, author contributions, prevalent keywords and citation networks. Through advanced data manipulation, aggregation and clustering, we unveiled underlying patterns, correlations and insights embedded within the dataset.

Data Analysis and Visualization

We began by retrieving data through queries in the WoSCC electronic database by use of relevant search terms and filters. Subsequently, we applied data extraction techniques to collect pertinent metadata, including publication titles, abstracts, authors, affiliations, and citation counts. This raw data was then imported into Biblioshiny,[19] a web-based platform known for its

robust data visualization capabilities and integrated with the RStudio[20] interface for advanced analytical processing.

Within Biblioshiny, we conducted a thorough analysis covering various dataset dimensions, such as frequency of publication, geographic distribution, institutional affiliations, prevalent keywords and citation networks. Employing computational algorithms and statistical methodologies, we manipulated, aggregated, and clustered data to reveal underlying patterns and insights.

To further refine and visualize our findings, we utilized Microsoft Excel 2023 for additional data preprocessing tasks and to create custom graphical representations. These visualizations, along with analytical insights, were synthesized to construct a coherent narrative, elucidating the interconnections between variables and observed trends within the dataset. Ultimately, the synthesized findings, raw data and visualizations were systematically organized and deposited into the Open Science Framework directory (DOI: 10.17605/OSF.IO/6DCU4) to facilitate peer review and knowledge dissemination within the scientific community (available at https://osf.io/6dcu4/).

Results

Outputs

From 2014 to 2023, our analysis covered a significant body of literature, comprising 1,681 documents sourced from 618 distinct publications. These documents involved the contributions of 10,940 authors, indicating extensive collaboration within the research community. The analysis identified 3,734 unique author keywords, providing insights into the breadth and depth of topics explored. International collaboration was prevalent, with 30.81% of the documents involving co-authors from different countries, which highlights the global nature of scholarly collaboration in this domain. On average, each document received approximately 29.84 citations, reflecting the impact of the research output. The annual growth rate of publications stood at -12.86%, demonstrating a dynamic and evolving landscape of research activity. Furthermore, the average age of the documents was 4.42 years, suggesting the relevance and currency of the research literature, with an average of 9.68 co-authors per document.

Annual Scientific Production

Analysis of annual trends reveals a notable progression in research output within the field from 2014 to 2023 (Figure 2). The number of articles published has generally increased over the years, which indicates a growing interest and engagement among researchers. Starting with 103 articles in 2014, the output climbed to 98 in 2015, followed by a slight decrease to 97 in 2016. The upward trajectory resumed in 2017 with 140 articles, continuing with 142 articles in 2018, 155 in 2019, and 188 in 2020. The year 2021 saw a remarkable spike, with 210 articles published, followed by 236 articles in 2022. The highest output was experienced in 2023 with 286 articles.

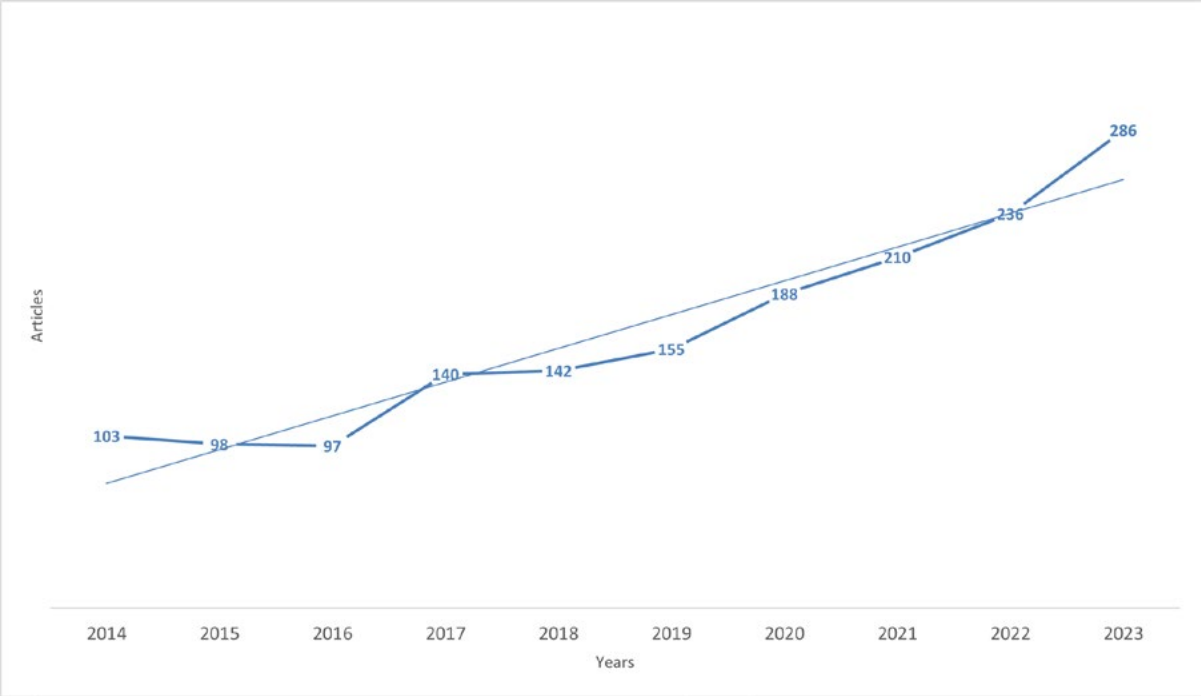

Figure 2. Annual scientific production from 2014 to 2023 as a function of the number of articles published.
Source: The authors (2024).

Citation Report

Based on the citation report obtained directly from WOSCC, we identified the contribution of the publishers to the evolving discourse surrounding the GS and AD (Figure 3). These publications have garnered considerable attention within the neuroscientific community, with a total of 28,158 citing articles identified. After taking into account self-citations, this number decreases slightly to 27,164, indicating the robust influence of the research output beyond its immediate sphere. Notably, researchers have cited these publications 50,140 times, demonstrating the significant impact they have had on advancing knowledge in the field. Excluding self-citations, the total number of citations stands at 43,292, with an average of 29.83 citations per item. Moreover, the H-index, a metric that quantifies the productivity and impact of a researcher's publications, is calculated at 97. This highlights the scholarly significance and influence of research conducted in this field. These statistics underscore the enduring relevance and impact of contributions to this critical area of scientific inquiry.

Most Relevant Affiliations

The analysis of affiliations reveals the institutions that have made significant contributions to research in the field. Leading this list is Columbia University with 153 articles, showcasing its prominent role in the advancement of knowledge in this area. Following closely are Washington University with 144 articles and Zhejiang University with 138 articles, indicating their substantial contributions to the field. The University of Rochester also stands out with 129 articles. Other notable contributors include Boston University with 123 articles, the University of Washington with 113 articles, and the University of Edinburgh with 105 articles. Additionally, institutions such as Oregon Health and Science University, with 104 articles, the University of Copenhagen, with 92 articles, and Rush University, with 91 articles have demonstrated noteworthy involvement. Each of these institutions has significantly contributed to the body

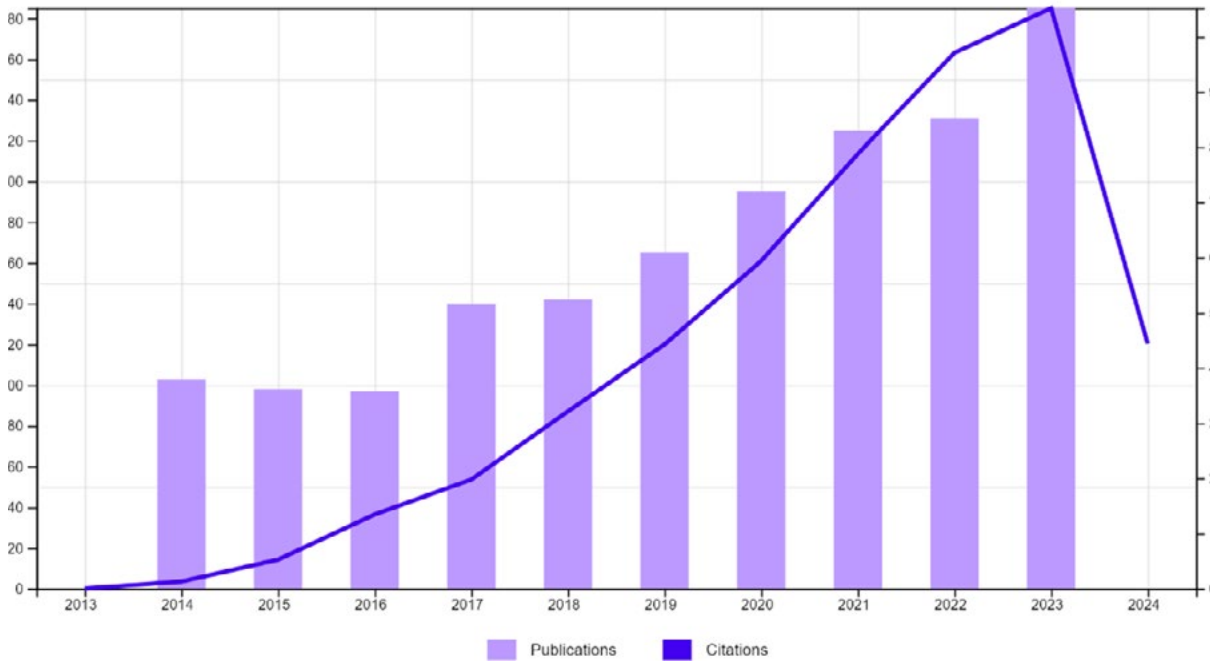

Figure 3. Annual scientific production from 2014 to 2023 as a function of publications and citations.
Source: The authors (2024).

of research. These findings underscore the collaborative efforts of institutions worldwide in advancing our understanding of this critical area of scientific inquiry.

Scientific Production and Impact by Country

The analysis of scientific production and impact in the field by country reveals a comprehensive view of global research efforts and their influence. Leading the list is the United States, with a staggering 3,578 publications, reflecting its profound contribution to advancing knowledge in this area. The United States also leads in citations, with a total citation count of 21,975 and an average of 46.00 citations per article, underscoring its significant influence in the field.

China follows closely with 1,723 publications, demonstrating its substantial engagement in research endeavors related to the topic. China also has a significant citation impact, with 4,825 total citations and an average of 16.60 citations per article, highlighting its emerging role as a major contributor to scientific discourse in this area.

The United Kingdom and Japan also made notable contributions, with the United Kingdom accounting for 527 publications and Japan with 569 publications. The United Kingdom has a total of 2,428 citations and an average of 41.20 citations per article, while Japan has 2,131 citations and an average of 17.50 citations per article. Germany, with 433 publications, has 818 total citations and an average of 13.40 citations per article.

Other notable contributors include France, with 449 publications and 2,148 total citations (35.20 average per article), Italy, with 438 publications and 1,135 total citations (17.20 average per article), and South Korea, with 482 publications and 1,465 total citations (19.00 average per article).

Countries such as the Netherlands, Spain, Canada, Sweden, and Australia also demonstrate significant impact and reach in scientific production and citations, illustrating a robust and

interconnected global scientific community actively contributing to advancements in understanding and addressing neurological disorders such as AD.

World Map of Collaboration by Country

The collaboration network analysis reveals a staggering 245 unique collaborations between countries, underscoring the global reach of research efforts in the field (Figure 4). Among these collaborations, prominent partnerships include those between the USA and China (61 collaborations), the USA and Denmark (45), and the USA and Canada (42). In addition, the United Kingdom has engaged in collaborative research with a multitude of countries, notably Germany (24 collaborations), Canada (23), and Spain (22). Other significant collaborations involve other partnerships of the USA, particularly with the United Kingdom (52 collaborations) and Australia (28). These collaborations highlight the importance of international cooperation in advancing scientific understanding and fostering innovation in the study of neurodegenerative diseases.

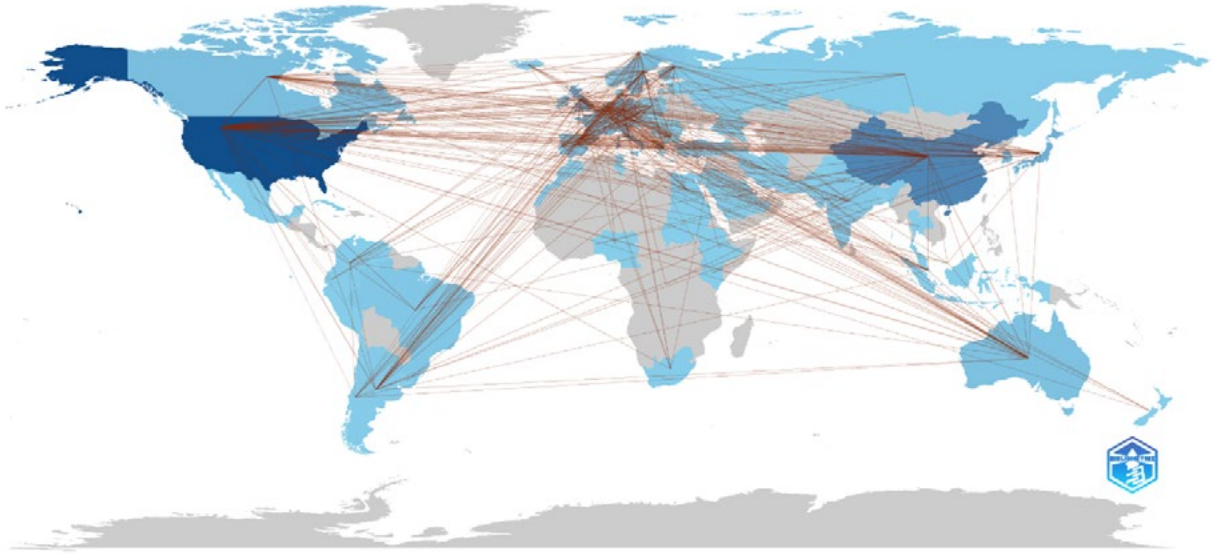

Figure 4. World map of collaboration by country.
Source: The authors (2024).

Co-occurrence network with thematic map (Authors' keywords)

The co-occurrence network analysis based on authors' keywords reveals distinct thematic clusters that contribute to our understanding of the GS and AD (Figure 5). Among the identified clusters, the most prominent include "glymphatic system," "Alzheimer's disease," and "dementia," indicating their pivotal roles in connecting other keywords within the network. Notably, the "perivascular spaces" cluster exhibits dense connections among keywords within this group. In addition, keywords such as "magnetic resonance imaging," "tau" and "aging" demonstrate significant importance in the network structure. Moreover, keywords like "cerebrospinal fluid," "magnetic resonance," and "meningeal lymphatic vessels" remain integral components of the thematic network, contributing to the comprehensive understanding of the etiology and pathophysiology of AD. This analysis provides insights into the interconnectedness of key concepts and themes within the research literature, thus facilitating further exploration and investigation in this critical field.

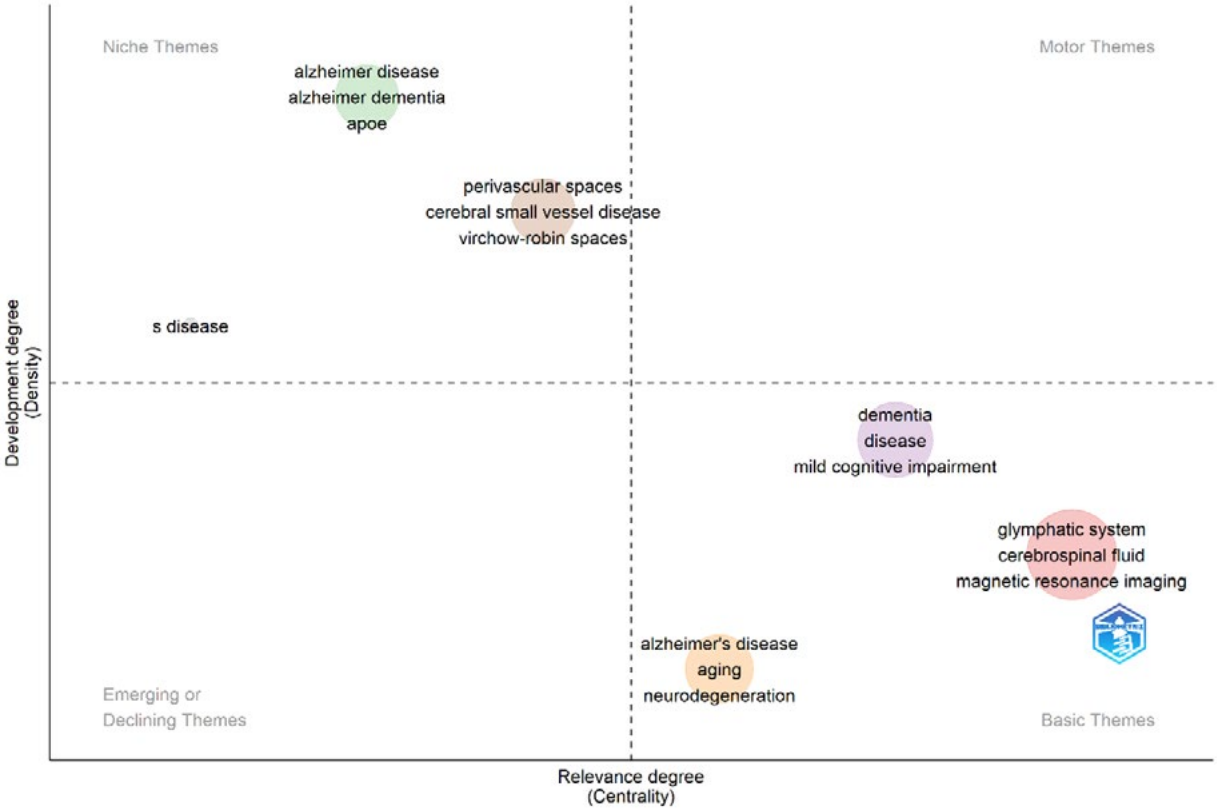

Figure 5. Co-occurrence network with thematic map (Author's keywords).
Source: The authors (2024).

Discussion

General Overview

In recent years, the increase in research efforts has underscored the significance of the GS within the neuroscientific community. Studies over the past decade have elucidated its role as a kind of "lymphatic system" in clearing metabolic waste and regulating water transport in the brain and the CNS21. While these findings are based on experiments conducted on animal models and studies utilizing MRI tracer-based techniques, the precise significance of the GS in waste clearance mechanisms within the CNS remains a topic of ongoing debate. This uncertainty is compounded by variations in imaging protocols,[22] including contrast agent injection protocols,[23] acquisition time points for dynamic contrast-enhanced scanning, and the specific anatomical structures targeted for measuring glymphatic flow.[24]

Our bibliometric analysis aimed to synthesize the research landscape, in particular the nexus between GS research and studies of AD pathology. However, despite our efforts, we encountered challenges in delineating clear correlations and drawing definitive conclusions. Methodologically, while we employed statistical techniques, the limitations of relying on the selected electronic database became apparent. The data retrieved, while extensive, may only partially capture the breadth and depth of research in this complex domain. Therefore, the interpretation of the findings is subject to caution.

Annual trends revealed a steady increase in research output, yet questions persist regarding the quality and rigor of these studies. Citation analysis, while indicating impact, also rais-

es concerns about self-citations and potential biases in citation practices. Furthermore, our analysis revealed an almost complete absence of low-income countries at the forefront of GS studies, despite the estimated higher number of individuals with dementia in such places. This disparity highlights issues of accessibility and resource allocation in global research endeavors.

The collaboration network analysis hinted at global cooperation in GS and AD research but also exposed gaps and disparities in research collaboration, particularly in regions with limited resources or restricted access to cutting-edge technology. The disparity in research capacity between low and high-resource countries, coupled with the prevailing global trend in health research, is a matter of concern. Therefore, the necessity for research collaboration between these two groups is crucial.[25] Thematic analysis of author's keywords provided some insights but also highlighted the fragmentary nature of research efforts, comprising disparate clusters that lacked cohesive integration.

Furthermore, it has been speculated that impaired GS function contributes to the progression of neurodegenerative disorders and emerges as a pivotal factor in their etiology.[26] While research linking the GS to AD is notable, the exploration of potential influences from other pathological processes, such as Parkinson's disease and other forms of dementia, remains limited.[27] This observation underscores the need for further investigation to broaden our understanding of the intricate interplay between the GS function and various neurological disorders.

Implications for clinical practice and clinical research

The discovery of the GS carries profound implications for both clinical practice and research in AD, which is characterized by the accumulation of toxic protein aggregates, such as Aβ plaques and tau tangles, within the brain.[28] Importantly, the blood-brain barrier (BBB) and blood-cerebrospinal fluid barrier (BCSFB) act as significant impediments to the clearance of these pathological proteins, exacerbating the progression of the disease14. Understanding the pivotal role of the GS in waste clearance from the brain opens avenues for innovative strategies to enhance drug delivery and facilitate the removal of toxic proteins associated with Alzheimer's.[29] Leveraging this system as a pathway for drug administration could significantly enhance the efficacy of therapeutics by bypassing traditional barriers that hinder their access to the brain. Furthermore, in clinical practice, the adoption of glymphatic-targeted therapies could transform the treatment landscape for Alzheimer's patients, offering more effective and precisely targeted treatment options that could lead to improved outcomes and enhanced quality of life.

As individuals age, the natural decline in glymphatic flow and clearance activity becomes increasingly evident, contributing to various health issues30. Research indicates a significant association between this decline and disrupted sleep patterns, such as insomnia and sleep apnea.[31] In addition, neurological disorders marked by cognitive decline, such as AD and other forms of dementia, have been linked to impaired glymphatic function.[14] Understanding these associations can have profound implications for clinical medical practice. Interventions to preserve or enhance the glymphatic function may offer novel therapeutic avenues for addressing sleep disorders and cognitive decline in aging populations. Moreover, monitoring glymphatic activity could potentially serve as a biomarker for early detection and monitoring of neurological diseases, facilitating timely interventions and improving patient outcomes.

Chronic nighttime insomnia and heightened daytime sleepiness are hallmark symptoms of AD[32,33] and are often correlated with the severity of cognitive decline. However, the exact

contribution of disrupted sleep patterns to either triggering the onset of AD or emerging as symptomatic of the disease remains elusive and warrants further inquiry, particularly in clinical research settings.[34] Notably, levels of soluble Aβ in the brain's interstitial fluid fluctuate daily in conjunction with the natural sleep-wake cycle.[35] Furthermore, sleep deprivation has been shown to markedly increase susceptibility to Aβ plaque formation, as observed in studies involving both animal models and human subjects.[36,37] Understanding the relationship between sleep disturbances and AD pathogenesis is crucial for developing effective therapeutic strategies. Insights into how sleep quality influences the accumulation of pathological proteins in the brain can inform interventions to mitigate the risk of AD development or slow its progression. Moreover, unraveling the mechanisms underlying the link between sleep deprivation and Aβ plaque formation could lead to novel approaches for preventing or treating AD-related cognitive decline.

Measuring the function of the GS in clinical settings poses significant challenges, particularly due to the limitations inherent in MRI technology. MRI provides a non-invasive method to visualize and potentially quantify the dynamics of fluid movement within the brain,[38] which is crucial for assessing the GS. However, the resolution of this technology may not fully capture the microscale fluid movements that are essential for a detailed understanding of glymphatic functionality. Moreover, the slow and subtle nature of glymphatic flow, combined with the potential discrepancies introduced by contrast agents used in some MRI studies, complicates the accurate detection and quantification of glymphatic activity.

Furthermore, the variability in physiological conditions among individuals, such as differences in age, circadian rhythms and neurological health, adds another layer of complexity to the standardization of measurement protocols across diverse patient populations. Analyzing MRI data to correlate fluid dynamics with glymphatic function requires sophisticated techniques and models, posing a barrier in routine clinical practice.[39] Despite these challenges, MRI remains one of the most promising tools for the study of the GS due to its non-invasive nature and the amount of information it can provide. Continued research and technological developments are expected to enhance the sensitivity and specificity of MRI techniques for more effective glymphatic assessment in the future.

Recommendations for Future Research

Several key areas warrant further exploration in the field of GS research and its implications for AD. Firstly, future studies should focus on elucidating the mechanistic underpinnings of the relationship between disrupted glymphatic function and AD pathogenesis. This includes studying the molecular pathways involved in regulating glymphatic flow and clearance activity, as well as how alterations in these processes contribute to the accumulation of pathological proteins such as Aβ and tau.

Furthermore, conducting longitudinal studies to understand the temporal dynamics of glymphatic dysfunction in the context of AD and other neurodegenerative diseases is crucial. These studies, which would involve tracking glymphatic activity in individuals at different stages of cognitive decline, could offer valuable insights into the role of glymphatic dysfunction as a predictive biomarker for AD onset and progression. In addition, exploration of the potential therapeutic interventions that modulate glymphatic function to prevent or decelerate the progression of AD could have a significant impact on the field.

In addition, given the complex interplay between the glymphatic function, sleep disturbances and cognitive decline, interdisciplinary approaches involving neurology, sleep medicine and geriatrics are essential for advancing our understanding of these relationships. Collaborative efforts between researchers from different disciplines can facilitate the development of comprehensive treatment strategies that target both glymphatic dysfunction and sleep disturbances in AD patients.

Among these areas, neuropsychology plays a critical role. Analyzing cognitive abilities through comprehensive cognitive testing can significantly contribute to tracking, identifying and deepening our understanding of the relationships between the GS and AD.[40,41] The application of scales and neuropsychological tests has proven instrumental in investigating cognitive declines and their progression.

Expanding on these recommendations, future research should also explore the impact of lifestyle interventions, such as exercise and dietary modifications, on glymphatic function and AD pathogenesis. Research on how lifestyle factors influence glymphatic activity and cognitive outcomes could provide valuable insights into the development of personalized preventive strategies for individuals at risk of AD.

In addition, the role of advanced neuroimaging techniques, such as MRI and positron emission tomography (PET), is crucial in providing non-invasive methods for assessing the glymphatic function *in vivo*. Integrating these imaging modalities with biomarker analyses and clinical assessments could enhance our ability to diagnose and monitor glymphatic dysfunction in AD patients, ultimately informing the development and evaluation of targeted therapeutic interventions.

Conclusion

Our study sheds light on the evolving landscape of glymphatic system research and its relevance to Alzheimer's disease. International collaboration and key publications are driving advancements in understanding AD pathology. Future research should elucidate the link between disrupted glymphatic function, sleep disturbances and the progression of AD. Interdisciplinary approaches are crucial for the development of effective treatment strategies. This analysis informs future research directions and underscores the importance of addressing neurodegenerative diseases.

Acknowledgements

We gratefully acknowledge the financial support provided by the Coordination for the Improvement of Higher Education Personnel (CAPES), Brazil (Grant No. 88887.841787/2023-00), the Foundation for Research Support of the Federal District (FAPDF), Brazil (Project No. 00193-00002602/2022-69, Grant No. #623/2022), and the Foundation for Scientific and Technological Enterprises (FINATEC), Brazil (Notice DPG/UnB 005/2024).

Conflict of interests

The authors declared no potential conflicts of interest with respect to the research, authorship and/or publication of this article.

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BJHBS

Analysis of the most commonly used clinical protocols in regenerative endodontic treatment

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Abstract

Introduction: Regenerative endodontic treatment (RET) does not yet have a well-established clinical protocol. Objective: The aim of this study was to carry out a scoping review of the RET clinical protocols described in the literature. Methodology and resources: The question used was: "What are the differences among published RET protocols and which are the most widely used?" The search was carried out in the Lilacs, BVS, PubMed, and Scielo databases. Studies reporting on pulp revascularization protocols published in the last ten years were included. Results and discussion: Seventy-four studies met the inclusion criteria. Most of the studies used sodium hypochlorite (NaOCl) as an auxiliary substance, either alone or in combination with other substances; however, the concentration of NaOCl used in the protocols varied greatly (between 0.5% and 6%). In more than 90% of the studies, treatment was carried out in 2 or 3 sessions, with intervals between sessions that ranged from 24 hours to 4 weeks. The most used intracanal medication was triple antibiotic paste, followed by calcium hydroxide. Blood clots were the most used type of scaffold, mineral trioxide aggregate (MTA) was the most used material for cervical sealing, and composite resin was used for coronal shielding. Conclusions: Performing the technique over multiple sessions, using triple antibiotic paste as intracanal medication, blood clot as a scaffold, and MTA for cervical sealing are some of the most common characteristics and materials found in clinical protocols for RET.

Keywords: Clinical protocols; Regenerative Endodontics; Dental Trauma; Permanent Dentition.

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BJHBS, Rio de Janeiro, 2025;24(1):62-72
DOI: 10.12957/bjhbs.2024.85469
Received on 31/01/2025. Approved on 12/05/2025.

Introduction

The presence of pulp necrosis in teeth with incomplete root formation represents a clinical challenge for endodontic specialists. Following the occurrence of pulp necrosis, the development of the canal walls is interrupted, resulting in teeth with incomplete root maturation. These teeth are characterized by thin root dentin walls that are susceptible to fractures, even under normal physiological stress conditions, as well as a wide-open apex, which represents a complication for endodontic treatment.[1,2]

The conventional approach to these cases has been to apply the apexification technique. This technique can be carried out in two ways: the first and most widely used technique involves cleaning and filling the root canal with a temporary calcium hydroxide-based paste

to stimulate the formation of calcified tissue at the apex.[3,4,5] The second technique is carried out by placing an apical plug composed of mineral trioxide aggregate (MTA) to act as a barrier against the condensed gutta-percha.[6]

Regenerative endodontic treatment (RET), also called pulp revitalization or pulp revascularization, has emerged as an alternative technique for the treatment of pulp necrosis in teeth with incomplete root formation with the aim of improving their prognosis. The concept of "endodontic regeneration" is recognized by the American Association of Endodontists (AAE), regardless of whether the result of the application of the protocols is actually “regeneration” or “repair”.[7,8] This therapy can be conducted in two different ways: through the cultivation of stem cells and their subsequent transplantation into the root canal or through the stimulation of free cells with chemotactic capacity.[9] For a long time, it was believed that the RET technique was ideally and practically restricted to young patients; however, as its use has expanded, it has also been applied to permanent and fully developed teeth.[10]

Several successful RET clinical protocols have been reported in recent years. A consensus exists as to the general principles of treatment, such as elimination of the infection, followed by the application of an intracanal medication, creation of a scaffold, and prevention of reinfection by sealing above the clot, as well as coronal shielding.[11] However, no standard protocol exists for this technique, which may inhibit its use.[9]

Bearing in mind that — although all RET protocols have the common objectives of disinfecting the root canal system and creating an environment that promotes the growth and differentiation of mesenchymal cells, as well as restoring function —, the technique’s protocols are not yet fully established,[12] the aim of this study was to carry out a scoping review of the clinical RET protocols described in the literature.

Methodology

The question that guided this study was: “What are the differences among the published RET protocols and which are the most widely used?”. The studies included were selected using the PCC (Population, Concept and Context) mnemonic strategy, as recommended by the Joanna Briggs Institute (JBI) protocol. The population was defined as immature and mature human permanent teeth with pulp necrosis; the concept was defined as pulp revascularization protocols; and the context assigned was the differences between pulp revascularization protocols and which are the most used in immature and mature necrotic permanent teeth, published in the last 10 years.

The search was carried out in June 2023 on the Lilacs, Virtual Health Library (VHL), Pubmed and Scielo databases, using the following terms for the search strategy: “regenerative endodontics”, “pulp revascularization”, “tooth root” and “dental pulp necrosis” (Table 1). Platform filters were used so that only studies published in the last ten years were included. Duplicate studies were removed using EndNote Web.

The studies selected included randomized clinical trials; cross-sectional studies; longitudinal studies; and case reports on pulp revascularization protocols. Theses, literature reviews, dissertations, monographs and publications that could not be accessed were excluded. The title, abstract and type of study were screened by two independent examiners using the Rayyan web application. The selected abstracts were then screened again by two independent examiners who read the files in full. In cases of disagreement, a third examiner was consulted.

Regarding the general characteristics of the articles, the following data were extracted: year of publication, population and sample size (when applicable), type of study, objective and results. With regard to the specific characteristics of the clinical revascularization protocol described, the following data were extracted: auxiliary chemicals, number of sessions, interval between sessions, intracanal medication/disinfection, framework, cervical sealing and coronal shielding. These data were grouped to identify the general and specific information related to each study, as recommended by the Joanna Briggs protocol (2015). The data collected were analyzed and interpreted based on previously established criteria.

Table 1. Number of studies found in each database.

Database	Search strategy	Studies
BVS	(regenerative endodontics) AND (pulp revascularization) AND (tooth root) AND (dental pulp necrosis)	36
BVS	(regenerative endodontics) AND (pulp revascularization) AND (tooth root) OR (dental pulp necrosis)	131
BVS	(regenerative endodontics) OR (pulp revascularization) AND (tooth root) OR (dental pulp necrosis)	345
SCIELO	(regenerative endodontics) AND (pulp revascularization) AND (tooth root) AND (dental pulp necrosis)	0
SCIELO	(regenerative endodontics) AND (pulp revascularization) AND (tooth root) OR (dental pulp necrosis)	4
SCIELO	(regenerative endodontics) OR (pulp revascularization) AND (tooth root) OR (dental pulp necrosis)	12
PUBMED	(regenerative endodontics) AND (pulp revascularization) AND (tooth root) AND (dental pulp necrosis)	11
PUBMED	(regenerative endodontics) AND (pulp revascularization) AND (tooth root) OR (dental pulp necrosis)	475
PUBMED	(regenerative endodontics) OR (pulp revascularization) AND (tooth root) OR (dental pulp necrosis)	585
LILACS	(regenerative endodontics) AND (pulp revascularization) AND (tooth root) AND (dental pulp necrosis)	6
LILACS	(regenerative endodontics) AND (pulp revascularization) AND (tooth root) OR (dental pulp necrosis)	9
LILACS	(regenerative endodontics) OR (pulp revascularization) AND (tooth root) OR (dental pulp necrosis)	30
	TOTAL	976

Source: The authors (2024).

Results

The chosen search strategy resulted in 976 articles (Table 1). Of these, 394 duplicates were removed. The remaining 582 files were screened by reading the title, abstract and type of study, as a result of which 91 articles were selected. Out of these, 17 articles were excluded because they did not describe a clinical protocol for pulp revascularization. Finally, 74 articles were included in the review (Figure 1)

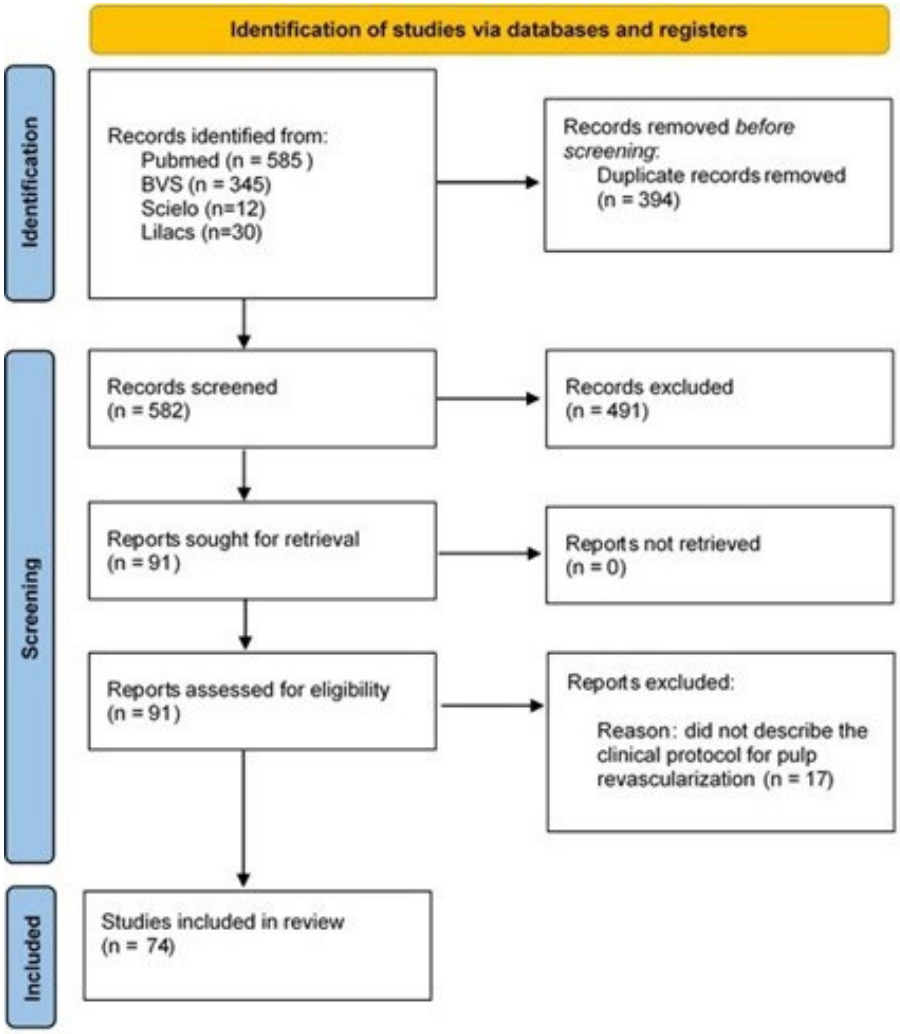

Figure 1. Flow diagram of the search.
Source: The authors (2024).

Analysis of the general characteristics of the selected studies in terms of year of publication found that around 62% of the studies were published in the last five years. With regard to the type of study, case reports and case series accounted for 74.3% of the publications. Prospective or retrospective clinical studies accounted for around 12.2% of all publications and their samples ranged from 10 to 116 treated teeth. Randomized clinical trials accounted for 13.5% of the studies and their samples ranged from 15 to 66 patients (Table 2).

With regard to the specific characteristics described in the RET protocols in terms of the auxiliary chemical substances used, the authors chose to use only hypochlorite as an irrigating solution in 13.5% of the articles. Other auxiliary chemical substances were mentioned during the chemical decontamination procedure, either in combination with hypochlorite or as a substitute. Among the substances most used in combination with hypochlorite was saline solution, found in 27% of the studies. In addition to this combination of saline solution and hypochlorite, the use of ethylenediaminetetraacetic acid (EDTA) was found in 27% of the articles. Some authors used only sodium hypochlorite and EDTA (18.9%) and a combination of sodium hypochlorite, saline solution, and chlorhexidine was cited in two articles, to which EDTA was also added in two studies (Table 3).

Table 2. General characteristics of the articles

Variable	Results
Year of publication 2013 to 2017 2018 to 2023	 28 (37.8%) 46 (62.2%)
Type of study Case reports or case series Prospective or retrospective studies	 55 (74.3%) 9 (12.2%)
Randomized clinical trials	10 (13.5%)

Source: The authors (2024).

Table 3. Specific characteristics of the RET protocols described

Features	Number of studies (%)
Auxiliary chemicals	
NaOCl only	10 (13.5%)
NaOCl + saline solution	20 (27.0%)
NaOCl + saline + EDTA	20 (27.0%)
NaOCl + EDTA	14 (18.9%)
NaOCl + saline + chlorhexidine	2 (2.7%)
NaOCl + saline + chlorhexidine +EDTA	2 (2.7%)
NaOCl + chlorhexidine	1 (1.4%)
NaOCl + other substances	3 (4.1%)
Saline only	2 (2.7%)
Sodium hypochlorite concentration	
NaOCl 1.0%	9 (12.2%)
NaOCl 1.5%	15 (20.2%)
NaOCl 2.5%	17 (23.0%)
NaOCl 5%	5 (6.7%)
NaOCl 5.25%	19 (25.7%)
Other concentrations (0.5%, 2%, 3%, 4%, 6%)	11 (14.9%)
Number of sessions	
Single session	2 (2.7%)
Two sessions	40 (54.0%)
Three sessions	32 (43.3%)
Interval between 1st and 2nd sessions	
Four weeks	18 (25.0%)
Three weeks	26 (36.1%)
Two weeks	12 (16.6%)
A week or less	9 (12.5%)
Not informed	7 (9.8%)
Interval between 2nd and 3rd sessions	
24 hours	10 (31.2%)
From 48 to 96 hours	8 (25.0%)
One week	9 (28.1%)
Two weeks or more	3 (9.4%)
Not informed	2 (6.3%)
Intracanal medication	
Metronidazole + minocycline + ciprofloxacin	34 (46.0%)
Metronidazole + ciprofloxacin	9 (12.2%)
Other combinations of antibiotics	10 (13.5%)
Calcium hydroxide only	13 (17.5%)
Combinations with calcium hydroxide	4 (5.4%)
Did not inform or did not use	4 (5.4%)

Source: The authors (2024).

Table 3. Specific characteristics of the RET protocols described (cont.)

Features	Number of studies (%)
Frame type	
Blood clot only	43 (58.1%)
PRF only	17 (23.0%)
PRP only	4 (5.4%)
Clot + PRF or PRP	6 (8.1%)
Other variations	4 (5.4%)
Cervical sealing	
Traditional MTA	40 (54.0%)
White MTA	16 (21.6%)
White MTA + collagen matrix	6 (8.1%)
Other materials	11 (15.0%)
No cervical sealing	1 (1.3%)
Coronary shielding	
Composite resin	32 (43.2%)
IVC + composite resin	21 (28.4%)
CIV only	15 (20.3%)
Other materials	5 (6.8%)
Not informed	1 (1.3%)

Source: The authors (2024).

In one article, chlorhexidine was used together with sodium hypochlorite, and in another article, together with EDTA. The following combinations were also used in only one publication each: sodium hypochlorite and hydrogen peroxide; sodium hypochlorite, saline solution, EDTA and distilled water; sodium hypochlorite, saline solution, EDTA, chlorhexidine, 5% sodium thiosulfate, 5% Tween 80 and 0.07% soy lecithin. In two publications, only saline solution was used as an auxiliary irrigating substance (Table 3).

The concentrations of sodium hypochlorite used in the protocols described showed a predominance of the 5.25% concentration, which was used in 25.7% of the publications. A 2.5% concentration was used in 23% of the studies, a 1.5% concentration in around 20%, and a 1% solution was mentioned in around 12% of the publications. Sodium hypochlorite at 5% was mentioned in 6.7% of the articles. The 3% and 0.5% concentrations were used in 4 studies each, while the 2%, 4% and 6% concentrations were mentioned in only one study each. Five studies tested two of the hypochlorite concentrations mentioned above. Two studies did not use sodium hypochlorite, and one did not state the concentration used (Table 3).

With regard to the number of sessions carried out, most studies described the procedure as being conducted in two (54%) or three sessions (43.2%). In 25% of the studies, the interval between the first and second sessions lasted four weeks. Three-week intervals were mentioned in 36.4% of the articles, 16.6% preferred a two-week interval and 11% chose to wait just one week between sessions. In only one study did the authors opt for a 48-hour interval (Table 3). Among the articles where the protocol was carried out in three sessions (32 studies), the 24-hour interval was the most used, being mentioned in 31% of the studies. The one-week interval was the second most cited (28.0%). Intervals of 48 hours, 72 hours or 96 hours were mentioned in 25% of the studies. Another 9.3% chose to wait two weeks or more. Two studies did not report the length of the second interval (Table 3).

The most prevalent procedures described used triple antibiotic paste (metronidazole, minocycline and ciprofloxacin) as intracanal medication, being present in 46% of the articles. The use

of doxycycline as a substitute for minocycline was noted in 2.7% of the studies, while clindamycin, cefaclor, spiramycin and amoxicillin were used as substitutes for minocycline in one article each. Use of a double antibiotic paste (metronidazole and ciprofloxacin) was recommended in 12.1% of the articles. In one publication, double antibiotic paste and zinc oxide were used in combination, while a combination of tetracycline and cortisone was used as medication in another study (Table 3).

Calcium hydroxide was used as an intracanal medication in 17.5% of the publications, as well as in association with 2% chlorhexidine in 2.7% of the studies. The combination of calcium hydroxide, 0.12% chlorhexidine and zinc oxide was used in only one article. Odontopaste, which is a combination of calcium hydroxide, clindamycin hydrochloride 5% and triamcinolone 1%, was also used in one study. Other disinfection methods that do not qualify as intracanal medication were mentioned, including photoactivated light and a perovskite laser, which were mentioned in one article each (Table 3).

With regard to the framework used in the regenerative procedure, blood clot stimulated by over instrumentation was chosen in the vast majority of protocols. In 43 studies (58.1%) only blood clot was used; in four studies, the clot was supplemented with fibrin-rich plasma (PRF); in one study there was a combination of clot and platelet-rich plasma (PRP); and in another the authors used a combination of blood clot and Gengigel. PRF and PRP were also used by themselves, PRF in 23% of the studies and PRP in 5.4%. Other scaffold variations were mentioned in the studies, including the use of collagen as a scaffold in two studies (2.7%), while hydroxyapatite and amniotic membrane were used in one study each (Table 3).

Another aspect subject to analysis was the cervical sealant used. MTA was the most commonly used material for this purpose, with traditional MTA being used in 54% of the studies. White MTA was the sealant of choice in 21.6% of the studies and was used in conjunction with collagen in 8% of the studies. Gray MTA was used in one study. Biodentine and Iroot BP are examples of other materials used for sealing the cervical region, having been selected in 5.4% and 4% of the publications, respectively. Iroot BP was also used in combination with collagen in one of the cases described, as was BC Sealer. Cem cement was used in two studies and only one study did not use cervical sealing in its protocol (Table 3).

Finally, coronal shielding is the last stage of the revascularization protocol and in 43.2% of cases the restorative material chosen was composite resin, followed by the use of glass ionomer cement (GIC) in around 28.3% of cases. GIC alone was also frequently cited, with this material being used on its own in 20.2% of the studies. Composite resin and Cavit were the materials used in 4% of the studies. In only one case was composite resin used in conjunction with zinc oxide, and eugenol and amalgam were also used in one study. In one of the publications, the authors did not inform which material was used for the shielding (Table 3).

Discussion

The number of studies included in this review and the predominance of case reports are evidence of the increasing popularity of the RET procedure. In addition, the progressive increase in the number of studies has promoted experimentation with new tools and materials, reflecting advances in protocols.

When analyzing the specific characteristics of the protocols mentioned in the studies, it can be seen that sodium hypochlorite (NaOCl) was used as the irrigating solution in more than 97% of

the RET protocols included. It is the main and most widely used irrigating solution substance for intraradicular canals, due to its antimicrobial activity and ability to dissolve tissue, as well as its deodorizing, whitening and lubricating action.[13] However, only 13% of the studies used only NaOCl; other auxiliary chemicals were mentioned during the chemical decontamination procedure.

Among the substances most commonly used in combination with hypochlorite is saline solution, which was found in 27% of the studies. The use of saline solution as an irrigant reduces the likelihood of allergic reactions to NaOCl and its interactions with other substances used in the treatment; however, this agent does not have any chemical disinfection properties, it acts solely mechanically and is susceptible to contamination.[14]

EDTA was also used in some protocols, either in addition to the combination of saline solution and hypochlorite (25.6%) or with NaOCl alone (18.9%). The use of EDTA is common in dentistry and it is the most widely used chelator, used to dissolve inorganic material. In regenerative procedures, it is capable of increasing the adhesion and differentiation of dental pulp stem cells and neutralizing the cytotoxic effects of sodium hypochlorite.[15,16]

Chlorhexidine was also added to the disinfection protocol in combination with hypochlorite, serum and/or EDTA in five of the studies analyzed. Chlorhexidine is one of the substances that has been mentioned in regenerative endodontics protocols, and has also been widely used in conventional endodontics, since it has broad-spectrum antibacterial activity and high substantivity, as well as the ability to suspend debris, although it has the disadvantage of not acting in tissue dissolution.[17]

Another important piece of information about the disinfection stage of the canals refers to the concentration of sodium hypochlorite used; among the studies analyzed, this concentration varied between 0.5% and 6%. In the protocols described, around a quarter of the studies used a hypochlorite concentration of 5.25%, which can be justified because of its greater antibacterial efficacy in comparison to other concentrations.14 A 2.5% concentration was used in 23% of the studies, a 1.5% concentration in 20%, and a 1% concentration was mentioned in 12% of the publications. The choice of lower concentrations of sodium hypochlorite is aimed at reducing its cytotoxic effect.[14]

With regard to the number of sessions performed, a significant predominance of the cases described procedures that lasted two or three sessions, indicating a preference for an interval when using intracanal medication in the RET procedure. This pattern is in line with the findings of Botero *et al.*,[18] who demonstrated a 33% success rate for single-session RET, compared to a 71% success rate when there was a break in the procedure.

The protocols that were conducted in multiple sessions showed variations in the length of each interval. Regarding the interval between the first and second sessions, which represents the period of exposure of the root dentin to intracanal medication, around two thirds of the protocols suggested an interval of four (25%) or three weeks (36%). It is important to note that prolonged exposure of root dentin to intracanal medication can result in changes to its structure, increasing susceptibility to fractures due to excessive demineralization and degradation of the collagen present in the dentin.[16]

The articles that opted to carry out the protocol in three sessions had a second interval between the procedures, now with the framework in the root canal. In these cases, more than 80% of the studies suggested that this interval should be less than 7 days, with a 24-hour

interval being the most used (31%). This interval was commonly used by the authors as a safety interval, prior to definitive restoration of the element, during which X-rays were taken to check for signs of interference in the procedure.[19,20,21]

With regard to intracanal medication, most authors (46%) used the triple antibiotic paste containing metronidazole, minocycline and ciprofloxacin. The current literature cites the triple paste recommended by Hoshino *et al.*[22] as the main intracanal medication in RET.[23,24,25] This combination of antibiotics demonstrates high antimicrobial efficacy and, when used in revascularization protocols, produces remarkable results in terms of the thickening of dentin walls.[25,26] Some authors made changes to the formulation of the triple antibiotic paste, either by the removal of minocycline or its replacement by other antibiotic agents, such as doxycycline, clindamycin, cefaclor, spiramycin and amoxicillin. This adjustment was probably motivated by the darkening of the dental crown associated with the use of minocycline.[24,25]

Calcium hydroxide was used as an intracanal medication in 17.5% of the publications, as well as being used in association with other agents, such as chlorhexidine, zinc oxide and clindamycin hydrochloride. Calcium hydroxide is an alkaline substance with a high pH level, which, when it comes into contact with fluids, dissociates into calcium and hydroxyl ions, providing it with antimicrobial properties. Due to these characteristics, it has been used as an intracanal medication in endodontics. In addition, evidence exists that suggests that its use in regenerative endodontics results in better apical closure performance compared to the use of triple antibiotic paste.[26,27,28]

With regard to the framework used in the regenerative procedure, the majority of protocols used blood clot stimulated by over instrumentation (scaffold) (71.6%). This phenomenon can be understood by the fact that it is a simple technique that does not require complex resources, just irrigants, intracanal medication and the framework formed by the blood clot stimulated in the apical region of the tooth. Although blood clot has been most commonly used, some authors have employed new techniques and a variety of framework materials, including PRF, PRP, collagen, hydroxyapatite and amniotic membrane.

With regard to the cervical sealer, MTA was the most common material used, either in traditional or white form. Since it is a biocompatible, bioinductive material with good marginal adaptation, MTA is an excellent choice for cervical sealing, as well as having a high pH, which gives it antibacterial action. One disadvantage of its use is that it can cause tooth discoloration.[27]

With regard to coronal shielding, which is the last stage of the RET protocol, the most prevalent restorative material was composite resin, followed by its use in conjunction with IVC. The materials used for this purpose need to maintain the seal of the root canals, as well as withstand the loads exerted on the restoration. Composite resins perform this function well, since they have excellent mechanical properties, such as surface smoothness and mechanical resistance, as do IVCs, which have a certain resistance, adhesion and marginal sealing.[29,30]

Finally, when analyzing the specific characteristics of the published protocols, no well-established protocol exists among the studies. Although all the techniques agree on disinfection without or with minimal mechanical instrumentation, the need for a framework, cervical sealing and coronary shielding, there is no standardization in terms of the number of sessions required, the interval between sessions, the chemical substances used for irrigation and the concentration of sodium hypochlorite. Therefore, new studies must be carried out in order to

ascertain the effectiveness of variations in protocols so that a single protocol can be better established, to continually seek the best clinical results.

In conclusion, studies show that RET is a procedure that is becoming more popular and new techniques and materials are being added to its protocol. Performing the technique over multiple sessions, using triple antibiotic paste as intracanal medication, blood clot as a scaffold, and MTA for cervical sealing are some of the most common characteristics and materials in the clinical protocols for RET.

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Brazilian Journal of Health and Biomedical Sciences
Paper submission

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As part of the submission process, authors are required to check off their submission's compliance with all of the following items, and submissions may be returned to authors that do not adhere to these guidelines.

The submission has not been previously published, nor is it before another journal for consideration (or an explanation has been provided in Comments to the Editor).

The submission file is in OpenOffice, Microsoft Word, or RTF document file format.

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The text is single-spaced; uses a 12-point font; employs italics, rather than underlining (except with URL addresses); and all illustrations, figures, and tables are placed within the text at the appropriate points, rather than at the end.

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the name of each author with their affiliation in this particular order: first name, abbreviated middle names, last name. Department (or service). Course. University (or institution). City, state/province/ territory, country.

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Types of papers

1. Original papers: Papers resulting of original research. Maximum of 5,000 words (excluding abstract and references) and five images or tables. Maximum of 40 listed references. They must be submitted in the following format:

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tain the essential information in the paper, but cannot be just a patchwork of sentences from it. It must be succinct and direct, highlighting what is most important in the full text in order to encourage a full reading. In the conclusion, all results must be related to the objectives of the study. The discussion must assert the contribution of the results to the body of knowledge about the subject of research.

Keywords: three to six terms related to the subject must be given, separated by semicolons, according to MeSh (Medical Subjects Headings) for English.

Full text

Introduction: it must be short and present the purpose (context and justification) of the study, including a short review of relevant studies about the subject, mentioning any recent progress, and referencing just what is appropriate.

Methodology and resources: this section must briefly present all the information needed for other researchers to replicate the study. Adopted procedures must be clearly described, as must the analyzed variables and tested hypotheses. Definitions must be given whenever necessary. Population, sample, and measurement instruments must be described and information about data gathering and processing must be given. If possible, validity scores must be included. Methods and techniques used must be duly detailed, including statistic methods. New or substantially modified methods must be described, with a justification for its use and mention of its limitations. Research ethics must be observed. Authors must explicitly state that the research was done within ethical standards and with the approval of an ethics committee.

Results: this section must be a concise report of all new information found, with minimum personal bias and judgment. The data must be presented in a logical sequence, starting with the most important information. Data from tables and images must not be repeated, but briefly referred to. It must state the significance of the new data and the relevance of the new findings in relation to established theories and to scientific literature. In this section must also be mentioned the limitations of the present work, as well as its implications for future research. Finally, conclusions must be included in this section, always related to the initially stated objectives.

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Tables and/or images: up to a maximum of five, including the authorship and/or source.

Tables: must be created in dedicated software, such as Excel. The width must be proportional to one page in the current layout. The font must be Arial, size 9, single space. Tables must be imported to and submitted in a text file: .doc/.docx (Microsoft Word), .rtf (Rich Text Format), or .odt (Open Document Text). They must be assigned a number in ascending order and receive a title and/or subtitle explanation. They must also be referenced within the text. The content of a table must not replicate that of an image nor vice versa. Their numbers must be assigned according to the order in which they are referenced in-text. All abbreviations must be explained with a legend below the table. There must be the source from which the table was extracted and/or the authorship of it, this information must be written below the table, after the legend for the abbreviations, if any.

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2. Clinical cases:

Case report: usually it describes one to three patients or a family case. The text must be up to 2,000 words long,

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