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Editorial

In the next year, 2022, we are planning to return to our in-person activities. After a long period in remote work, this brings us great breath and hope for better days. Mass vaccination is a gold medal won despite many difficulties and obscure obstacles, in opposition to our principle of taking science above beliefs, empty opinions, without ambiguity, and fake news.

This issue of our journal crowns this hopeful return with interesting articles such as the epidemiological study of hypertensive elderly people, risk factors associated with many morbidities including respiratory syndrome caused by viruses; association between smoking and COVID 19, a subject always highlighted by the current and importance of the topic; well-designed protocols for clinical studies with vibration exercises; infectious diseases in intensive care units, a matter of extreme relevance, given the exponential increase in these patients caused by the pandemic; in addition to studies in genetics and biochemistry, important analytical parameters in studies of cancer and metabolic alterations related to smoking, a great epidemic of our times.

Finally, review articles with relevant topics in tropical infectious disease and cancer. We close with a clinical case to sharpen our reasoning and bring a light and stimulating learning experience.

A good read for everyone, happy 2022.

Fabricio Borges Carrerette
Associate Editor

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Original article

Clinical and epidemiological profile of elderly patients seen in a hypertensive emergency at a public hospital in the state of Rio de Janeiro - Hypertensive emergency in the elderly

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Abstract

Introduction: Hypertensive emergencies (HE) are clinical entities characterized by an acute and significant increase in blood pressure (BP) associated with severe symptoms that evidence target organ damage. Objective: To analyze the clinical and epidemiological profile of elderly patients treated in a hypertensive emergency at a public hospital in the state of Rio de Janeiro. Method: This study is prospective and descriptive. Information was collected from August to October, 2020 using medical records and processed using the Statistic Statsoft program. Pearson's test was performed for univariate analysis. Results: The sample consisted of 109 patients. The average age found was 73 years old, with a predominance of males (69.72%) and brown skin (36.69%). Approximately 72.48% of the patients had a history of Systemic Arterial Hypertension (SAH), however, (50.46%) reported that they did not regularly use antihypertensive medication. Target organ injuries were ischemic stroke (58.71%), followed by acute coronary syndrome (24.77%), hemorrhagic stroke (9.17%) and edema acute lung disease (7.33%). Regarding the BP values found at admission, the mean systolic pressure was 205.0 mmHg, while the mean diastolic pressure was 127.0 mmHg. Conclusion: Ischemic stroke was the most frequent HE in the sample. It is necessary to put in place measures to prevent risk factors associated with SAH, as well as to control blood pressure levels to reduce the number of consultations due to hypertensive emergencies and other complications of cardiovascular diseases.

Keywords: Emergency; Hypertension; Prevalence.

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Introduction

Systemic arterial hypertension (SAH) is a multifactorial clinical condition characterized by a sustained increase in blood pressure levels ≥ 140 and/or 90 mmHg. It is often associated with metabolic disorders, functional and/or structural alterations of target organs, being aggravated by the presence of other risk factors (RF), such as dyslipidemia, abdominal obesity, glucose intolerance and diabetes mellitus (DM).[1,2]

Several cohort studies have shown that the increase in BP provides similar risks to those shown for CAD (coronary artery disease) and CVA (stroke) for the incidence of other cardiovascular outcomes. These include heart failure (HF), with and without preserved ejection fraction (EF), atrial fibrillation, valvular heart disease, peripheral arterial disease, chronic kidney disease (CKD), dementia, and Alzheimer's disease.[3]

Data on the prevalence of SAH in the country tend to vary according to the methodology and casuistry used. Considering the measured BP and the use of antihypertensive medication, the percentage of adults with BP greater than or equal to 140 per 90 mmHg reached 32.3% (95% CI 31.7-33.0). It was found that the prevalence of AH was higher among men, and, as expected, it increased with age by all criteria, reaching 71.7% for individuals over 70 years of age.[4]

The high prevalence leads to complications in target organs, fatal and non-fatal, such as: heart: coronary artery disease (CAD), heart failure (HF), atrial fibrillation (AF) and sudden death; brain: ischemic (EVA) or hemorrhagic (AVEH) cerebrovascular accident (CVA), dementia; kidneys: CKD that may progress to the need for dialysis therapy; and arterial system: peripheral arterial disease (PAD).[3]

Abrupt and severe elevation of AH, usually defined by diastolic pressure values above 120 mmHg characterizes the hypertensive crisis (HC).[5] This clinical condition is classified as hypertensive urgency (HU) when there is no damage to target organs and hypertensive emergency (EH) when there is a risk to the patient's life evidenced by target organ damage, and that is why the measures used to combat high blood

pressure levels must be immediate, in minutes or a few hours, requiring the use of fast-acting drugs and by the parenteral route.[6]

HC must be differentiated from hypertensive pseudocrisis, which is accompanied by a marked increase in BP, triggered, in most cases, by the abandonment of drug treatment in chronic hypertensive patients, but also by anxiety, pain, and exacerbated use of salt in food. The outstanding clinical evidence in hypertensive pseudocrisis is the absence of signs of rapid target organ deterioration. In this case, there is no need to use medications for rapid BP control, just the use of symptomatic medication and the introduction of chronic antihypertensive drugs, analgesics, tranquilizers or even rest.[7]

Recognizing the importance of the subject and considering its high incidence in urgent and emergency services, this study aimed to analyze the clinical and epidemiological profile of hypertensive emergencies in a hospital located in Nova Iguaçu, RJ, Brasil.

Material and methods

The present study was approved by the Ethics and Research Committee of Universidade Iguaçu under CAAE number 14126119.4.0000.8044.

A prospective study was carried out to analyze the medical records of patients with elevated diastolic blood pressure levels > 120 mmHg and target organ lesions, treated at the emergency department of the General Hospital of Nova Iguaçu, in the city of Nova Iguaçu, RJ, Brazil, from August to October, 2020.

Patients over 60 years of age, of both genders, with or without prior comorbidities and diagnosed with HE were included in the study. Patients under 60 years of age and with HU were excluded from the study.

The prevalence of HS was estimated, as well as an analysis of variables: gender, age group, skin color, history of SAH, treatment performed, previous pathologies and symptoms. For this purpose, we performed a univariate analysis, using the Pearson test (X2) to observe possible associations between dependent and independent variables.

To identify factors associated with SAH, univariate logistic regression analyzes were performed, with associations considered statistically significant when $p \leq 0.05$. Statistical analysis was performed with the aid of the statistical application Statistic Statsoft.

Results

The sample included 109 patients. The mean systolic BP was 205 mmHg, the mean diastolic was 127 mmHg.

From the outline of the epidemiological profile of the sample, shown in Table 1, the age group ranged from 60 to 90 years, with a predominance between 71-80 years (55.05%). It was found that the average age was 73 years.

Males became preponderant (69.72%) in relation to females (30.28%). It was noted that brown individuals had higher incidences of HE (36.69%), followed by black individuals (32.11%) and later white individuals (31.19%). It was found that 72.48% of the patients had a history of SAH, however, 50.46% of the sample did not undergo antihypertensive therapy, as shown in Table 1.

Among the patients who underwent antihypertensive treatment (49.54%), 48.14% were undergoing single drug therapy, while 51.85% were undergoing combined therapy. Of the drugs used as monotherapy, it was noted that 61.5% of the patients used drugs of the angiotensin II receptor antagonist (ARA), 19.23% diuretics, 11.53% angiotensin-converting enzyme inhibitors (ACEI) and 7.69% beta-blocker.

Complaints and symptoms presented by elderly patients on admission were shown in Table 2, and could be a sign mentioned more than once. The most prevalent signs and symptoms were: cecreased level of consciousness, hemiparesis and/or hemiplegia, aphasia and commissure deviation, configuring cerebrovascular manifestations as the most prevalent in the period (67.88%), followed by anginal pain and dyspnea, characterizing cardiovascular manifestations.

Regarding past pathological history, the most prevalent cited was SAH itself (72.48%), followed by diabetes mellitus (27.52%), ischemic and hemorrhagic stroke (19.26%) and acute myocardial infarction (AMI) (16.76%).

Target organ damage was diagnosed through clinical history and laboratory tests. 58.71% of the patients had ischemic stroke, 24.77% acute coronary syndrome, 9.17% hemorrhagic stroke and 7.33% acute lung edema.

Discussion

The present study showed alarming data on the blood pressure control of the elderly with antihypertensive drugs, since most patients (50.46%) did not take drug therapy to control the disease, thus, we could raise the hypothesis that the poor AH control caused the patients to evolve with HS.

Checchi et al, reported that 30% of the patients in their sample did not adhere to antihypertensive treatment, either because of adherence to drug therapy, or because of inappropriate lifestyle or because

the culture of drug use is not yet fully disseminated in the elderly population.[8]

The main consequence of the lack of adherence to treatment is the lack of AH control and, therefore, the increase in target organ damage (LOA) and cardiovascular morbidity and mortality (CV). However, it is noteworthy that there was no significant association between the use or not of regular medication and risk for HE. Such analysis can be a data collection bias, as many patients may omit or not regularly use the medication.

AH is the most prevalent non-communicable chronic disease among the elderly.[9] There is a direct and linear relationship between BP and age, with the prevalence of AH being greater than 60% in the age group above 65 years.[10] The Framingham Study points out that 90% of individuals with normal BP up to 55 years of age will develop hypertension throughout their lives.[11] In Brazil, the prevalence of hypertension in individuals over 60 years of age is about 65%.[12]

It is noteworthy that in this study, care was more prevalent in males, however, for Franco and Faustino,[13] HC was more prevalent in females. In the literature, some articles report that among women older than 75 years the prevalence of SAH can reach 80%. Therefore, blood pressure values vary according to age and sex, in addition to being influenced by the medications used, associated chronic diseases and lifestyle changes.

The most predominant age group of HS cases was between 71 and 80 years, in self-declared brown or black individuals. It is known that black individuals are more susceptible to arterial hypertension, although such mechanisms are not fully elucidated. Studies conducted in the United States of America[14] show that the prevalence and incidence of HA is at least twice as high among blacks as among whites. These differences become even more marked with regard to the more severe forms and complications. Brazilian studies also show that the prevalence of AH is higher among blacks.[9,11,13]

With regard to therapy, combined therapy was predominant (50.93%), and when we analyzed monotherapy, the drug most used by the study population was the class of angiotensin II receptor antagonists, also observed in the study of Franco and Faustino,[13] who report that the preference of losartana potassium for captopril, as the latter causes more side effects, such as the well-known cough. Losartan Potassium is a more selective drug, with reduced side effects.[13,14]

Our data reveal that SAH and diabetes were the comorbidities most cited in the previous pathological history, these being the most prevalent chronic pathologies in Brazil in the elderly population,[10] and the overlap of the two pathologies is still a risk factor for cardiovascular diseases.[7] The profile The clinical spectrum of hypertensive emergency proved to be very well defined, with the vast majority of patients being admitted to the emergency room with neurological symptoms and final clinical diagnosis of ischemic stroke, also observed in other studies.[7,9-12]

Conclusion

Our study reveals that the clinical-epidemiological profile of hypertensive emergencies in the elderly shows the black and male population as the most affected. Furthermore, the high number of patients who do not properly adhere to treatment substantially increases the demand for hospital service in the presence of a hypertensive emergency, highlighting the need for public policies aimed at preventing health problems related to non-control of SAH, which per hour increases the demand for hospital services even more, given the need to monitor sequelae caused by lesions in target organs.

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Original article

Smoking and COVID 19: Analysing this controversy in a Brazilian COVID-19 Reference Centre

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Abstract

Introduction: Due to the COVID-19 pandemic, it is extremely important to determine the risk factors that define patients who are more susceptible to the severe form of the disease; however, the observation of supposedly protective factors is also of great relevance. Smoking has been the subject of controversy as to whether it is a protective factor or a risk factor for COVID-19. Objective: To assess how smokers behave within the context of the COVID-19 pandemic. Patients and methods: The participants in this study were a spontaneously recruited sample from the Rio de Janeiro State University COVID-19 Reference Centre, between March and May 2020. All patients underwent clinical, laboratory, and nasal swabs for the Sars-Cov-2 PCR investigation. Whenever it was the necessary case, patients were referred to hospitalization. Results: A total of 4,636 patients with suggestive symptoms of COVID were evaluated. There was 230 (4.9%) smokers in this group; there is a 10.3% smoking prevalence in the state of Rio de Janeiro as described in 2018. A number of 2,246 patients (48.6% of the total sample) were diagnosed with COVID-19, only 82 of these (3.7% of the total positive COVID) were smokers. Only 1 (0.01%) of the smokers with COVID-19 needed hospitalization. As far as the assessed symptoms, smokers showed fewer symptoms during the disease. Conclusion: The study suggests that smokers have fewer symptoms (mild or asymptomatic symptoms) and that there is a need to expand specific testing for that group.

Keywords: COVID-19; SARS-CoV-2; Smoking; Risk factor.

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Introduction

The COVID-19 pandemic that started in December 2019, in China is caused by the new coronavirus known as Sars-Cov-2.[1] The viral transmission occurs through droplets dispersed in the air and the disease incubation varies from 2-14 days. The virus spectrum is wide, from asymptomatic forms to severe pulmonary conditions.[2] Due to its high degree of transmissibility, it is important to determine the risk factors for the susceptibility to acquiring the disease and its severe form, as well as those that possibly confer protection.

Several risk factors have been identified since the beginning of the pandemic, namely: age > 60, the presence of comorbidities such as systemic arterial hypertension, diabetes mellitus, chronic cardiovascular diseases and chronic respiratory diseases.[3,4] In addition to these, chronic kidney diseases, history of transplantation, immunosuppression, including HIV despite CD4+ and the use of immunobiologicals are also described.[5]

As for smoking, this was initially understood as a risk factor, as it doubles the risk for infection by the influenza virus and it is a risk factor for bacterial pneumonia.[6] Smoking has been considered a risk factor for mortality, but the frequency of smoking patients with active COVID infection has been controversial.[7] Smoking is responsible for around 200 thousand deaths per year in the USA and it is a risk factor for various diseases, including cardiovascular, pulmonary, and neoplastic diseases which are risk factors for the COVID-19. Nevertheless, the assessment of smoking patients and its relationship with the COVID-19 has been the focus of interest.[8]

In a cross-sectional study, we evaluated the relationship between the COVID-19 and smoking in a sample obtained from spontaneous subjects for the assessment of symptoms and diagnosis of the COVID-19.

Methods

Study procedures

A cross-sectional study carried out between March 19th and May 18st 2020, at the Rio de Janeiro State University Reference Centre for screening patients and health professionals with symptoms of COVID-19. These 4.636 individuals who pursued assistance were recruited and all of them reported the COVID-19 symptoms within a time spam of up to 10 days before the evaluation. All patients were invited to participate in the study and signed the CAAE Informed Consent Form numbered - 30135320.0.0000.5259.

The patients were clinically evaluated and their epidemiologic data was collected (Table 1), such as smoking history and smoking load and they were referred to nasal swab collection for Sars-Cov-2 Specific real-time reverse transcription polymerase chain reaction (rt-PCR) performed with the nasopharyngeal swab technique, at each nostril, up to the nasopharyx (NPhS-PCR). rt-PCR assays were performed using commercial kits or designed rt-PCR kits (Biomanguinhos Fiocruz, Rio de Janeiro, and Molecular Biology, Institute of Paraná, Paraná) approved by Brazilian Vigilance (ANVISA). Patients who presented relevant respiratory symptoms or oxygen saturation below 95% underwent a chest ultrasound with the use of the GE Logico E PRO equipment and whenever they needed, they were hospitalized. The COVID-19 diagnosis was confirmed in the presence of the PCR positive nasal swabbing.

Statistical analysis

Statistical analysis was performed using the statistical program SPSS 2017. Age, gender, and smoking load are described as average and standard deviation. The differences between the groups were evaluated by T Student, ANOVA, and the Chi-Square model whenever it was suitable. Statistical significance was defined by p lower than 0.05.

Results

A total of 4,636 patients were assessed for the diagnosis of the COVID-19, 4406 (94.3%) non-smokers, and 230 (5.6%) smokers. The COVID-19 diagnosis was confirmed by NPhS PCR in 2,246 patients (48.4% of total patients), of whom, 2,164 (96.3%) were non-smokers and 82 (3.7%) were smokers. Of the 4,406 non-smoking patients, 2,164 (49.1%) presented a positive NPhS-PCR, 456 (10.3%) were non-conclusive and 1,786 (40.5%) negative (Table 2).

Regarding the 230 smoking patients, 82 of them (35.6%) had the diagnosis confirmed, 24 of them (10.4%) had a non-conclusive diagnosis and 124 of them (53.9%) negative. When comparing non-smokers with smokers

Table 1. Epidemiological data for smoking and non-smoking patients from the total sample

Profile	Non-smokers (n=4,406)	Smokers (n=230)
Male (n%)	1265 (28.7%)	84 (36.5%)
Age (n +/- DP)	40.4 +/- 10.6	42.8 +/- 11.8
Caucasians (n%)	2007 (45.5%)	98 (42.6%)
Education > 12 years (n%)	3010 (68.3%)	131 (56.9%)

Source: The authors (2021).

Table 2. SARS-COV-2 nasopharynx swab PCR result for smoking and non-smoking patients

	Non-smokers	Smokers	Total
Positive	2,164	82	2,246 (48,6%)
Non-conclusive	456	24	480 (10,3%)
Negative	1,786	124	1,910 (41,2%)
Total	4,406 (95,0%)	230 (5,0%)	4,636 (100%)

Obs.: The total number of smokers who sought the percent of diagnosis to perform the diagnostic investigation was lower in relation to the prevalence of smokers and also the frequency of diagnosis with p <0.001.

Source: The authors (2021).

regarding the positive PCR, we noticed a significant higher frequency among non-smokers (p=0.0001). Only one COVID-19 smoking needed hospitalization. The symptoms evaluated are described in Table 3, where the presence of smoking and non-smoking patients with positive PCR for Sars-Cov-2 is compared.

Discussion

The prevalence of smoking in the Rio de Janeiro state population is 10.3%. Our study pointed to a lower frequency of symptomatic patients with COVID who seek the diagnosis centres of COVID and these, which is already being presented also in the world literature. The percentage of positive COVID-19 smokers is lower than the percentage of smokers concerning the total sample (1,8% vs 46,7%). In addition, the frequency of symptoms in COVID smokers with positive RT-PCR was much lower than in non-smokers.

Vardavas et al, in a meta-analysis, concluded that smoking is possibly associated with a negative outcome in patients with the COVID-19.[10] Smoking seems to be a risk factor as it is associated with the increase of the expression of the gene of the angiotensin 2 converting enzyme (ACE2), which is fundamental for the entry of the virus into the cell.[11] The overabundance of ACE2 in the lungs of smokers may partially explain a higher vulnerability of smokers.[12]

In a recent document, the World Health Organization emphasized a possible relationship between smoking and a greater likelihood of developing the disease and death.[13] Some meta-analyzes have published a prevalence with a wide range of smokers among patients from 1.4 to 12.6%, with the prevalence pooled between 6.5 and 7.6%.[14,15] In our study 3.7% of the cases of COVID were smokers. However, when assessing the risk of smoking for death it becomes an independent and important variable. Some studies have shown that smoking is a well-established risk factor, with OR: 2.0 (95% CI 1.3-3.2) or OR 2.2 (95% CI 1.3-3.7).[16,17]

SARS-Cov-2 uses the angiotensin converting enzyme 2 receptor to enter the cell and there is evidence related to nicotine modulation in the expression of angiotensin converting enzyme 2 and its subsequent modulation of nicotinic acetylcholine receptors. The virus would alter the acetylcholine control in these receptors.[7] Cigarrete smoking up regulates the SARS-Cov-2 receptor ACE2 in humans and could increase the likelihood of being infected. On the other hand, increase expression of this enzyme could attenuate the risk of a devasting lung injury.[18]

The inflammatory process of smoking should be a risk condition for COVID. It is associated with the risk of mortality. Perhaps the lower frequency of smoking patients looking for the places of diagnosis of COVID are related to previous infectious processes, particularly by Influenzae, inferring a defense against coronavirus infection (crossed immunity) or the devaluation or lack of its symptoms that indicate the need for go to the doctor.[19] This can be identified in our article, bringing the need for comprehensive testing of the entire population due to the risk of asymptomatic carriers or with mild symptoms.

The main limitation of the study is the non-follow-up of patients who are positive in the COVID test and who were smokers and their clinical evolution. Another limitation is that the smoking status is self-reported.

Our study corroborates the findings of this group demonstrating that there was a lower demand for the screening service by smokers when compared to the prevalence of smokers in the state of Rio de Janeiro. This reinforce that is important to explore the interaction between smoking and COVID.

Table 3. Symptoms presented by smoking and non-smoking patients with positive rt-PCR for Sars-Cov-2

Symptom (n/%)	Non-smokers (n=2,164)	Smokers (n=82)	P
Fever	1458 (64.5%)	51 (45.5%)	0.059
Dyspnoea	618 (52.7%)	29 (34.1%)	**0.006**
Cough	345 (37.2%)	8 (13.1%)	**0.018**
Sneeze	1096 (56.0%)	45 (38.4%)	**0.017**
Sore throat	930 (49.6%)	33 (34.7%)	**0.001**
Myalgia	1541 (60.7%)	59 (43.3%)	**0.001**
Headache	1591 (56.2%)	61 (40.9%)	**0.002**
Anosmia	1070 (74.7%)	46 (61.3%)	**0.008**

Source: The authors (2021).

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Original article

WBVE in patients with COPD: A randomized trial protocol

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Abstract

Introduction: Patients with chronic obstructive pulmonary disease (COPD) have several impairments, reducing the quality of life (QoL). Pulmonary rehabilitation (PR) that does not exacerbate the disease, such as whole-body vibration exercise (WBVE), is recommended to improve patient outcomes. Objective: This study will describe a protocol for patients with COPD to assess respiratory parameters in two postures on a vibrating platform (VP). Methods: It will be a randomized controlled clinical trial with blind analysis. COPD patients will be allocated into three groups: Control Group (CG), Sitting Group (WBVE-S), and Standing Group (WBVE-ST). The intervention will be 6 weeks in alternating VP, with the same biomechanical parameters. Maximum inspiratory pressures (MIP), and maximum expiratory pressures (MEP), dyspnea, and QoL will be assessed. Discussion: Assess which posture will provide the best clinical results.

Keywords: COPD; Protocol; Exercise; Quality of life; Dyspnea; Respiratory muscle strength.

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Introduction

Chronic obstructive pulmonary disease (COPD) is one of the top five causes of morbidity and mortality worldwide.[1] Despite efforts, costs, and medical research around the world, COPD figures show a trend of continuous increase in mortality.[2] Exacerbations and comorbidities contribute to general severity in individual patients.[3] COPD is characterized by a reduction in airflow in the airways caused by an inflammatory response to inhaled toxins. This reduction leads to a significant decrease in muscle strength and endurance that can arise in relatively early stages of the disease, compromising the functional state and quality of life (QoL).[4] Treatment, pharmacological and non-pharmacological, is extremely important for the carrier of the disease. In this sense, pulmonary rehabilitation (PR) of COPD patients has emerged as a standard recommendation among non-pharmacological treatments.[6] Usually, a PR program has, among its objectives, to improve the symptoms of the disease, improve the QoL and promote the physical improvement of patients for activities of daily living.[7] However, patients with greater intolerance to exercise may not benefit from a traditional PR program, thus increasing the demand for health services.[9] An alternative intervention for PR exercises is the WBVE.[10] Studies in COPD patients have shown that WBVE has beneficial effects in improving exercise capacity, in addition to being easy, safe to perform, and cost-effective.[11] WBVE is an exercise modality in which the individual is exposed to mechanical vibrations produced on a vibrating platform (VP).[12] This vibratory stimulus is characterized by biomechanical parameters such as frequency, peak-peak displacement, and peak acceleration; and it can stimulate muscle spindles generating reflex activity similar to the tonic reflexes that cause involuntary muscle contractions triggered by monosynaptic drugs.[13] VP can be alternated (up and down oscillation on the opposite side), vertical (synchronous or tri-planar) and horizontal. WBVE can be performed with the individual standing (squatting) at the base of the VP[14] or sitting in a chair in front of the VP with feet at the

base of the VP. In individuals with COPD, WBVE can improve QoL[15] and functionality.[16] However, there are no standardized protocols to be implemented in these patients.[17] A common feature among studies involving individuals with COPD is the squat posture (patient standing with knee flexion at 1300) in PV.[18] As these individuals present worsening health status, some patients may not be able to maintain this posture and, consequently, will not receive the benefits offered by WBVE.[19] The present study aims to compare the clinical effects of the WBVE protocol in two different postures (squat and sitting in an auxiliary chair) on QoL, respiratory muscle strength, and dyspnea in patients with COPD.

Methods

This project was approved by the Certificate of Presentation of Ethical Appreciation (CAAE 49219115.3.0000.5259) of Hospital Universitário Pedro Ernesto (HUPE) and The Brazilian Registry of Clinical Trials (ReBEC RBR-72dqtm). The study was also registered in the procols.io platform (dx.doi.org/10.17504/protocols.io.376grre) and will follow the principles stated in the Declaration of Helsinki. All the participants of this work will sign a consent form.

Participants, interventions, and outcomes

Study settings

Outpatients of the Pulmonology Services of the *Hospital Universitário Pedro Ernesto* (HUPE), and *Policlínica Piquet Carneiro, Rio de Janeiro* of the *Universidade do Estado do Rio de Janeiro* (UERJ) will be recruited.

Inclusion criteria

Individuals of both sexes, aged 40 years or older, diagnosed with COPD based on the criteria established by the GOLD Document, patients with a stable disease with Forced Expiratory Volume in the First Second (FEV1) <50%, independent patients.

Exclusion criteria

Individuals with exacerbation the past 3 months; labyrinthitis; reported osteoporosis; other respiratory diseases; use of pacemakers; previous history of fractures and/or other orthopedic diseases submitted to surgeries with implantation of a metallic material; peripheral vascular disease and/or thromboembolism; decompensated cardiovascular disease; aneurysm; previous vitreous hemorrhage; malnutrition; a neurological disease that generates "fear" of VP movements; severe or disabling clinical disease at the discretion of the investigator; smoking and/or alcoholic individual.

Sample size

The sample size will be calculated using the quantitative formula of an infinite population[20] with the parameter "modified medical research council" (mMRC) from the article by[20] considering a standard deviation of 15.9 and a mean of 117 resulting in 25 patients for each group in this study. The formula for calculating sample sizes to describe quantitative variables in a population. n = sample size; Zα / 2 - critical value for the desired degree of confidence, usually: 1.96 (95%); δ - population standard deviation of the variable; E - standard error, usually: ± 5% of the proportion of cases (absolute precision), or ± 5% of the mean (1.05 mean).

Participant timeline

In figure 1, we show how patients WILL recruited for the protocol. After going through the eligibility process, we will do blind randomization using an opaque envelope, and define the 3 groups: Control Group (CG), Sitting Group (WBVE-S), and Standing Group (WBVE-ST). The next phase will be the comparison between the groups before the intervention and only then do we start the 6 weeks of intervention. At the end of this period, we will enter the last phase, which will be the reassessment between groups. The individual that develops a serious illness that limits participation in the study or with an adverse event to the WBVE will ask to give up.

Parameters at intervention with WBVE

WBVEG-ST and WBVEG-S patients will use alternating VP (*Novaplate* fitness evolution, DAF *Produtos Hospitalares Ltda, Estek, São Paulo,* Brazil). The biomechanical parameters used will be peak-to-peak displacement (DPP) of 2.5mm and frequency (*f*) of 25Hz. WBVE will and held once a week for 6 weeks. In each session, the individual will perform 5 bouts consisting of 1 minute with vibration, working time (WT), and 1 minute without vibration, rest time (RT). A supervisor will follow all the interventions to instruct the patient to report any discomfort.

Interventions

WBVEG-ST: Participants will stand on the base of the VP with their knees bent at 130° controlled by a goniometer[19] without shoes and with their hands resting on bars on the side of the VP.

WBVEG-S: Participants will perform the protocol while sitting in an auxiliary chair in front of the VP, with their hands on their knees bent at 130° with their elbows straight. This position facilitates the proper transmission of mechanical vibration throughout the patient's body.

CG: Participants will undergo initial clinical evaluations, which are the outcomes of this work, and will be instructed to continue their daily activities normally and not to participate in any other regular rehabilitation program for 6 weeks. After this period time, they will return to the final clinical evaluations.

Outcomes

Respiratory muscle strength: Three measurements of the maximum inspiratory pressures (MIP), and maximum expiratory pressures (MEP) will be performed, using the highest value obtained for analysis by manovacuometry (Murenas Produtos para Saúde Ltda, Brazil). These measurements will be performed before and after the first session and before and after the last session.[21]

QoL: Before the first and after the last intervention session, the St. Georges Respiratory Questionnaire (SGRQ)[22,23] will be used in the evaluation of the QoL.

Dyspnea: The modified Borg Scale,[24] *Medical Research Council (*MRC),[25] COPD Assessment Test (CAT),[26] and Subjective Effort Perception Scale for dyspnea (EPSE)[27] will be used to verify the exacerbations of the symptoms.

Data analysis

The Prism statistical program (GraphPad Inc. USA) will be used to perform the statistical analyses and the $p \leq 0.05$ will be considered significant. The intention-to-treat analysis will be performed including all participants in the analysis according to the original group allocation. Repeated measurement analysis of variance will be used to assess the difference between and within the group. The Bonferroni hoc test post will be used to compare the results.

Discussion

PR is one of the essential components of the non-pharmacological intervention in comprehensive COPD services,[13] including exercise.[18] The importance of prescribing physical activity to COPD patients is determined, among other factors, by (a) high morbidity and mortality associated with COPD;[16] (b) evidence that physical inactivity is associated with an increased risk of mortality and exacerbations;[16,17] (c) physical inactivity is associated with progressive exercise intolerance and muscle involvement;[18] (d) low levels of physical activity occur even in patients with mild COPD, suggesting the need for early intervention to reduce the risk of future comorbidities and, possibly, disease progression.[10,21]

An alternative type of exercise to be included in PR would be WBVE.[28] WBVE sessions show important positive responses for COPD patients. A diversity of protocols using different biomechanical parameters, as well as the positioning of the individual, working time (WT) and rest time (RT) between vibratory stimuli have been described.[17]

Regarding the positioning of the individuals on the VP, authors commonly consider the standing posture however, research is sparse regarding the advantages of this posture when compared to others.[29] The present study aims to perform a comparison of the findings related to respiratory muscle strength, dyspnea, and QoL with the individual in two positions, standing (squat) or sitting in an auxiliary chair. These results may provide evidence to support the beneficial effects of WBVE without the risk of causing the disease to exacerbate, and which patient positioning should adopt.

Braz Jr et al[15] reported that WBVE could be a safe and viable intervention in PR using the squat position protocol. Zhou J et al[11] suggested that WBVE could improve lung function and QoL in COPD patients about the change in FEV 1 (% predicates) and the SGRQ score. Using the protocol, low *f,* and, squat position [31], and Teixeira, et al [32], showed higher scores activity, impact, and in the total SGRQ domains, reflecting with this a greater disposition for daily activities with reduction of respiratory symptoms.

The importance of the current study is to present independent results for respiratory muscle strength, dyspnea, and QoL, and to be able to compare them between two positions on VP. This comparative result may show in which posture we will have a better response to the clinical conditions of that patient with COPD.

Limitations

This is a systematic review of the best available knowledge of the effects of physical exercise in patients with COPD with several protocols, and possible exacerbations that can show up. The potential prescription

and employment of physical exercise in selected COPD patients will require careful evaluation by multidisciplinary teams.

Declaration of conflicting interests

The author(s) declared no potential conflicts of interest for the research, authorship, and/or publication of this article.

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Original article

Multiresistance and endemic status of *Corynebacterium striatum* associated with nosocomial infections: A critical situation in ICU and varied wards of a tertiary care hospital, Rio de Janeiro metropolitan area, Brazil

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Abstract

Nowadays, *Corynebacterium striatum* has been reported as etiologic agent of mild to severe hospital-acquired infections, including patients undergoing endotracheal intubation with fatal outcome. A continuous survey of infections due multidrug-resistant (MDR) *C. striatum* in South American countries remains necessary. This retrospective and prospective study aimed to analyze clinical-microbiological features of *C. striatum* clinical isolates from patients attended at a Brazilian tertiary care hospital, during a nine-year period. *C. striatum* strains (n=130) were isolated from infected patients attended at twenty nosocomial wards, mainly of tracheal aspirate (n=53) and blood and/or intravenous catheter (n=21/n=13). Notably, original cases of nosocomial infections due to *C. striatum* were verified in children making use of invasive devices, including one fatal neonatal case, in addition to a fatal respiratory infection in patient with cystic fibrosis, as well as urinary tract infections of kidney transplant recipients. Most of *C. striatum* strains expressed MDR profiles (88.46%). Emergence of vancomycin (1.90%) and linezolid (7.41%) resistance was verified among MDR and non-MDR-strains. In conclusion, endemic condition with wide dissemination among hospital wards of varied types of infections due to MDR and non-MDR *C. striatum* strains expressing heterogenic virulence potential and genetic features may occur in hospital units.

Keywords: *Corynebacterium striatum*; Multidrug resistance; nosocomial infections; virulence; endemic.

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Introduction

Currently, a growing number of reports have demonstrated *Corynebacterium* spp. as etiologic agents of a variety of infectious processes in both immunocompromised and immunocompetent patients. At least fifty *Corynebacterium* species have been recognized of medical, veterinary, or biotechnological relevance, including diphtheria toxin (DT)-producing *Corynebacterium diphtheriae, Corynebacterium ulcerans, Corynebacterium pseudotuberculosis, Corynebacterium belfanti* and non-DT-producing *Corynebacterium* spp. participants of human microbiota.[1-3] The increasing number of reports concerning infections related to different and new *Corynebacterium* species were favored by genotyping and taxonomy studies, laboratorial identification techniques, and/or immunocompromised patients survival conditions.[4-6] Although, diphtheria vaccination programs have been implemented in many countries, diphtheria outbreaks, atypical cases of diphtheria, and cases of invasive infections, including immunized individuals caused by DT-producing and non-DT-producing *C. diphtheriae* and *C. ulcerans* zoonotic pathogen have been reported, including Brazil.[6,7] Consequently, microbiologists must not promptly discard colonies of irregular Gram-positive rods (IGPRs) from cultures, even when grown associated with one or more poten-

tially pathogenic clinical isolate, especially in cases of infections in tropical and/or developing countries.[1,6,8,9]

Corynebacterium striatum was firstly considered as part of the skin and mucous membranes of healthy normal persons unable of causing disease. However, further studies have increasingly recognized *C. striatum* as etiologic agent of a variety types of infections that can range from mild signs and symptoms to severe illness and even death of patients. *C. striatum* expressing multidrug-resistant (MDR) profiles have been also related to nosocomial infections and outbreaks in different countries, especially in long-term hospitalized patients with prolonged exposure to broad-spectrum antibiotics and admitted in intensive care units (ICUs) or surgical wards using continuous or prolonged medical devices and respiratory recuperation.[4,10,11] An increasing number of studies demonstrated variations in clinical sites among patients with *C. striatum* infections: bacteremia, sepsis and catheter-related infections,[4,12] endocarditis, meningitis,[13,14] septic arthritis,[15] osteomyelitis[16] and pulmonary infections.[4,12] *C. striatum* strains have also been identified as etiologic agents of breast abscesses,[6] skin lesions and surgical wounds,[17] urinary tract and intrauterine infections[18] including patients affected by AIDS and cancer.[6]

Multidrug-resistant (MDR) *C. striatum* strains have been increasingly reported as pathogens of nosocomial infections and outbreaks in different continents of industrialized and developing countries, including China, Japan, United States, Canada, Belgium, Italy, Spain, Tunisia, and Brazil.[19,20] During the year 2009, a nosocomial outbreak caused by *C. striatum* strains was initially verified in a Brazilian university hospital located at Rio de Janeiro metropolitan area.[1] Pulsed field gel electrophoresis (PFGE) analysis indicated the presence of four profiles, including two predominant and related MDR clones (PFGE-types I and II) that were mainly isolated from patients undergoing endotracheal intubation procedures from ICUs and surgical wards. Subsequently, cases of bloodstream and catheter-related infections caused by *C. striatum* isolates were progressively certified.[21]

Therefore, investigation of clinical, epidemiological, and microbiological features of nosocomial infections caused by *C. striatum* is necessary to prevent future problems and guarantee continued vigilance by researchers, bacteriology laboratory and medical professionals concerning community, nosocomial infections, and outbreaks due to MDR emergent pathogens.[8,9] The aim of this study was to assess the diversity, dissemination, and persistence of *C. striatum* strains isolated from patients attended in a Brazilian tertiary care hospital – located at Rio de Janeiro metropolitan area, during a nine-year period (2009-2018).

Materials and methods

Study design and origin of bacterial isolates

This retrospective and prospective investigation demonstrated and characterized the presence of *C. striatum* strains among clinical isolates recovered from patients with signs and symptoms of bacterial infections as part of medical care procedures in different wards, during a nine-year period (August 2009 – August 2018) of Hospital Universitário Pedro Ernesto (HUPE) – a tertiary care hospital of Rio de Janeiro State University – UERJ, located at the metropolitan area of Rio de Janeiro, RJ, Brazil.

Clinical strains of Irregular Gram-Positive Rods (IGPRs) were previously detected by using regular diagnostic cultures in the Bacteriology Laboratory (LABAC/UERJ) and routinely sent for further analysis in the Laboratory of Diphtheria and Corynebacteriosis of Clinical Importance (LDCIC/FCM/UERJ). All *Corynebacterium* strains were stored in Trypticase Soy Broth (TSB) with 20% glycerol at -80ºC in LDCIC collection.

Briefly, clinical strains of IGPRs were selected for further identification when cultures were grown in any quantity from normally sterile body fluid or when IGPR strains were isolated in significant numbers or in pure culture from other specimens obtained at clinical sites where infection was suspected. *Corynebacterium*-like (coryneform) colonies were selected for identification when grown in significant numbers (>15 colonies) or in pure culture from blood or catheter samples, as recommended by Maki's semi-quantitative method to distinguish infection (>15 colonies) from contamination of catheter-tips. Microorganisms were identified from the urine cultures and considered to be potential pathogens, as follows: bacterial growth $>10^4$ CFU $mL^{[-1]}$ as the only isolate; $>10^5$ CFU $mL^{[-1]}$ as the predominant isolate; $>10^3$ CFU $mL^{[-1]}$ in cases of nephropathies. Clinical samples yielding more than three organisms were regarded as contaminated and discarded in most opportunities, except in cases of urine samples of patients submitted to renal transplantation, with procedures established in accordance with LABAC and LDCIC units.[1,8]

This study was developed in compliance with the Brazilian Government's Ethical Guidelines for research

involving human beings (resolution of the National Health Council/Ministry of Health) and approved by the Research Ethics Committee of the Hospital University Pedro Ernesto of Rio de Janeiro city (CAAE 44674314.3.3001.5091). The consent to participate was not required because all the investigated *C. striatum* clinical isolates were taken as a part of standard care (diagnostic purposes) and no identifiable human data were used.

Culture conditions and identification procedures of *C. striatum* clinical isolates

Clinical isolates identified as IGPRs were inoculated onto Columbia agar base with the addition of 5% sheep's blood and incubated at 37 ºC in a 3-5% CO_2 atmosphere and monitored for 72 h. Phenotypic analysis of IGPRs clinical strains included colonial morphology, pigmentation, motility, hemolysis, lipolytic, catalase and DNase activities; CAMP reaction by using beta-lysin-producing. *Staphylococcus aureus* strain, among other screening, phenotypic and molecular procedures regularly achieved in LDCIC unit. During August 2009 – August 2018, identification and/or reidentification of *C. striatum* strains were done by phenotypic and molecular procedures, as previously described: (i) commercially available semi-automated identification API-Coryne System 3.0 (bioMérieux) with the API web decoding system (www.apiweb.biomerieux.com) (all possibility >90%); (ii) MALDI-TOF (Matrix Assisted Laser Desorption Ionization Time-Of-Flight, Bruker Daltonics™).[3] Each bacterial colony were tested in duplicate onto a 98-target plate to verify reproducibility and achieve proper identification. Identification criteria recommended by equipment manufacturer were as follows: score ≥2.000, species-level; 1.700 - 1.999, genus level ; ≤ 1.700, no identification; (iii) 16S rRNA and *rpoB* gene amplification and sequencing assays for one strain.[1,21] The 16S rRNA gene sequences were compared with National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov) using BLAST algorithm and Ribosomal Database Project (RDP-II) (http://rdp.cme.msu.edu/html). The *rpoB* gene sequences were compared at GenBank database.[1,21-23]

Antimicrobial susceptibility testing and characterization of MDR profiles

Antimicrobial susceptibility profiles were described by making use of the disk diffusion method in Mueller-Hinton agar supplemented with 5% sheep blood, according to CLSI document M20. Vancomycin susceptibility testing was interpreted in accordance with criteria defined for *Staphylococcus* spp. and *Enterococcus* spp.[24] The antimicrobial agents (Oxoid SA, Spain) tested included: penicillin (10 UI), ampicillin (30μg), cefotaxime (30 μg), imipenem (10 μg), erythromycin (15 μg), clindamycin (2 μg), gentamicin (10 μg), ciprofloxacin (5 μg), tetracycline (30 μg), rifampin (5 μg), linezolid (30 μg), vancomycin (30 μg). MDR profiles were defined as acquired non-susceptibility to at least one agent in three or more antimicrobial categories.[23,25]

Pulsed field gel electrophoresis assays

Thirty-seven *C. striatum* strains were previously submitted to analysis of genetic diversity by using Pulsed field gel electrophoresis (PFGE) assays, as previously described. PFGE profiles were characterized by roman numerals and subtypes were identified by roman numerals followed by a letter. The similarities were determined by Dice correlation coefficient. Similarity coefficient ≥85% were considered genetically related.[21]

Results

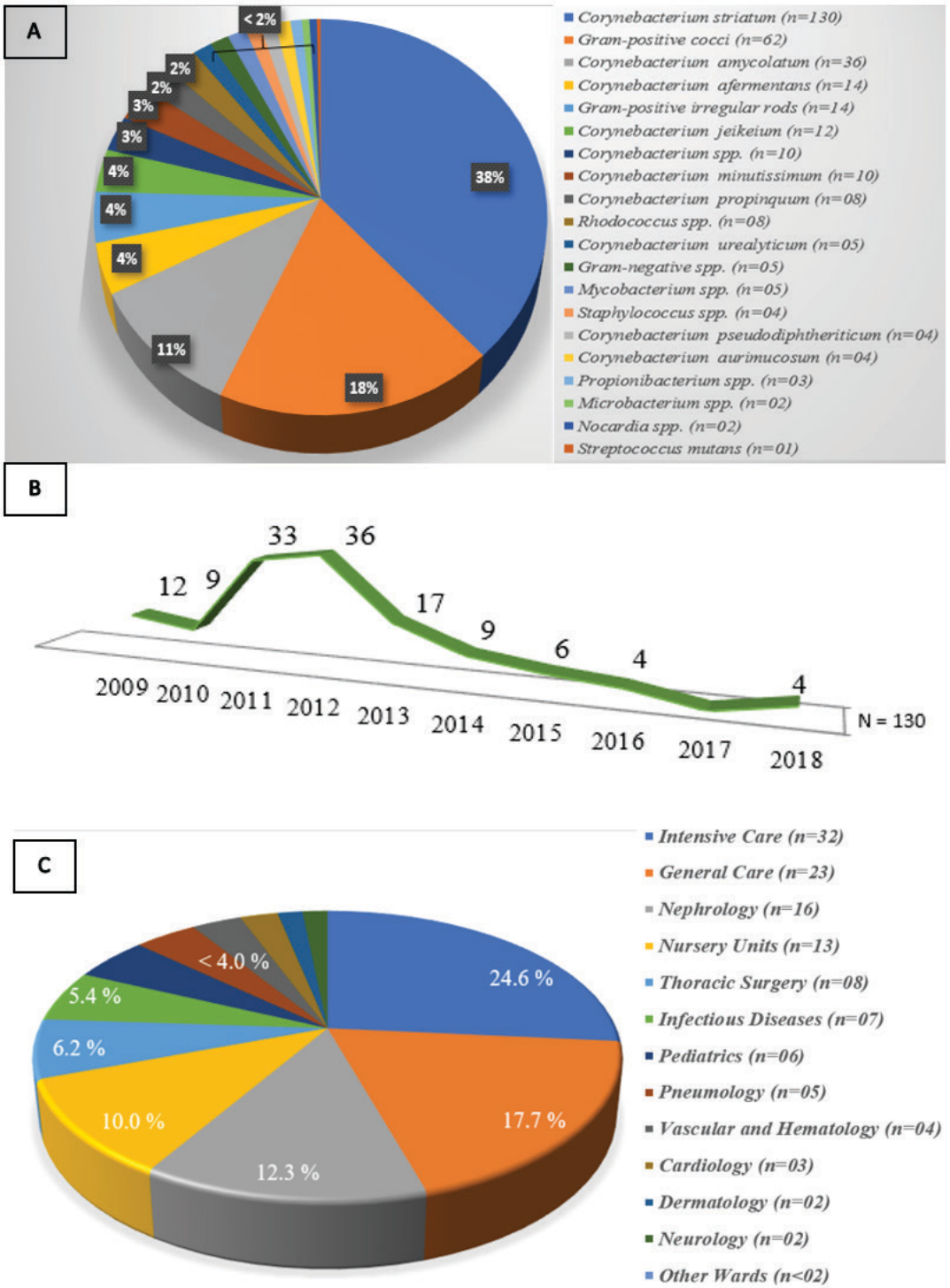
C. striatum and other *Corynebacterium* spp. isolated from clinical samples:

Corynebacterium of varied species and other Gram-positive and Gram-negative pathogens isolated from clinical samples by using routine diagnostic procedures in LABAC/UERJ and sent for further analysis in LDCIC/FCM-UERJ during and/or post nosocomial outbreak, were displayed in Figure 1. A total of 339 IGPRs strains were isolated from patients with signs and symptoms of infection as part of medical care procedures in HUPE/UERJ throughout the nine-year period of study (August 2009 - August 2018). Most of *Corynebacterium* clinical isolates were identified as *C. striatum* (n=130; 38.34%). However, other *Corynebacterium* species were isolated from clinical samples of infected patients at lower quantities (<10%), including *Corynebacterium amycolatum*, *Corynebacterium aurimucosum Corynebacterium propinquum*, *Corynebacterium afermentans*, *Corynebacterium urealyticum*, and *Corynebacterium jeikeium* (Figure 1A).

Number of *C. striatum* clinical strains isolated per year

The annual number *C. striatum* strains isolated from infected patients attended in HUPE/UERJ (August 2009 - August 2018) was displayed in Figure 1B.

Figure 1. *Corynebacterium striatum* **and other** *Corynebacterium* **spp. in addition to varied Gram-positive and Gram-negative pathogens isolated from clinical samples by using routine diagnostic procedures in LABAC/UERJ and sent for further analysis in LDCIC/FCM-UERJ during a nine-year period of study (A); number of** *C. striatum* **strains isolated from infected patients attended in HUPE/UERJ from August 2009 to August 2018 (B); and dissemination of** *C. striatum* **pathogen among hospital wards (C)**



Source: The authors (2021).

During the nosocomial outbreak (August 2009 – May 2010) *C. striatum* strains (n=15) were recovered from representative clinical sites of 14 hospitalized adult patients with signs and symptoms of bacterial infection: 50% male; age ≥50 years-old and 50% with informed fatal outcomes (n=07). Thereafter, a total of 115 *C. striatum* strains were isolated during the post-outbreak period (nine-year; May 2010 - August 2018): 2010 (n= 06), 2011 (n=33), 2012 (n=36), 2013 (n=17), 2014 (n= 09), 2015 (n=06); 2016 (n=04), 2018 (n=04). The highest number of *C. striatum* strains isolated from infected patients were detected during the years 2011 and 2012. A decrease in number of *C. striatum* clinical isolates initiated in 2013.

Dissemination of *C. striatum* pathogen among hospital wards

During the period from August 2009-August 2018, *C. striatum* strains were isolated from infected patients attended at different wards of a 600-bed university hospital, as shown in Figure 1C, Tables 1 and 2. Present study verified that 130 *C. striatum* clinical samples were isolated from patients attended over twenty distinct hospital wards, mostly ICUs (24.6%), General Care (17.7%), Nephrology (12.3%) and Nursery Units (10%). During the nosocomial outbreak period (August 2009-April 2010), *C. striatum* strains (n=15) were isolated from patients of five different hospital wards, but mostly (n = 10) from inpatients admitted to ICUs and surgical wards. Data revealed a subsequent increase of intra-hospital dissemination during the post-outbreak period in HUPE/UERJ. Interestingly, *C. striatum* strains were also related to cases of infections in Pediatric and Adolescent Care wards and neonatal ICU (n=09), and Cystic fibrosis (n=01) units.

Antimicrobial susceptibility analysis

Percentages of antimicrobial resistant *C. striatum* strains (n=130) isolated from varied clinical samples in HUPE/UERJ were shown in Figure 2A. Most of *C. striatum* clinical isolates (88.46%) from infected patients attended at different nosocomial wards expressed multidrug-resistance (MDR) profiles. *C. striatum* nosocomial clinical strains exhibited diverse resistance arrangements when tested for twelve antimicrobial agents used to treat Gram-positive infections. Higher number of resistant *C. striatum* strains were verified for clindamycin (88.75%), erythromycin (85.96%), ciprofloxacin (76.52%) and penicillin (76.52%), followed by trimethoprim-sulfamethoxazole (76.29%). *C. striatum* strains were also found resistant at lower rates to gentamicin (58.16%), rifampicin (35.40%), and tetracycline (10.38%). In the present study, emergence of linezolid-resistance (7.41%) and vancomycin-resistance (1.90%) was certified among *C. striatum* strains isolated from varied clinical sites of infected patients attended at different nosocomial wards.

Predominance and variability of PFGE-types among *C. striatum* clinical isolates

PFGE profiles presented by *C. striatum* strains isolated from different clinical specimens of infected patients attended in distinct hospital wards were shown in Table 1. Eleven PFGE profiles were verified among *C. striatum* isolates and designated I, Ia, Ib, II, V, VI, VIa, VII, VIII, IX and X. The PFGE - type I, subtypes - Ia, and Ib and PFGE - type II were previously considered genetically related, as well as PFGE - type VI and subtype - VIa: with ≥ 85% and ≥ 90% similarity coefficients, respectively. All *C. striatum* strains of PFGE-type I were characterized as MDR and found to express different MDR profiles. Interestingly, a diversity of PFGE profiles (n=10) were verified among MDR *C. striatum* strains isolated from different clinical specimens of infected patients attended in distinct hospital wards: I, Ia, Ib, II, V, VI, VIa, VII, VIII, and IX. *C. striatum* clinical isolates comprising PFGE-types III, IV, X were characterized as non-MDR.

From August 2009-April 2010, PFGE-type I was the most frequently genotypic profile observed among *C. striatum* strains isolated from infected patients (71.42%). *C. striatum* strains isolated from tracheal aspirate of patients with ventilator-associated respiratory tract infection or pneumonia were mostly of PFGE type I. *C. striatum* comprising PFGE-type I isolates were also isolated from bronchoalveolar lavage (BAL) (n = 1), cerebrospinal fluid (CSF) (n = 1), surgical wounds (n = 1), urine (n = 1) and blood (n=1) samples. Interestingly, analysis from Jan 2009-February 2013 showed that *C. striatum* strains were increasingly isolated from blood (n=13) and catheter (n=10) samples: 2009 (n = 02), 2010 (n = 04), 2011 (n = 10), 2012 (n = 06) and 2013 (n = 01). *C. striatum* comprising a diversity of genetically related and/or distinct PFGE-types (n=11) were isolated from patients with hematogenic infections: I, Ia, Ib, II; V; VI, VIa; VII; VIII; IX; X. PFGE profile I was also found predominant among patients with hematogenic infections.

Table 1. Pulsed-field gel electrophoresis retrospective analysis and clinical-microbiological features of *Corynebacterium striatum* strains (n=38) isolated from infected patients attended at the university hospital - HUPE/ UERJ, Rio de Janeiro, Brazil

PFGE-types-sub-types	Antimicrobial-resistance Profiles	Date/year	Number of strains			Clinical Samples Hospital	
			Total	Outbreak Aug 09 April 10	Post-Outbreak	Sites	Wards
I	MDR	2009, 2010, 2011, 2013, 2014	20	12	08	Tracheal aspirate, Bronchoalveolar lavage, Blood, Intravenous catheter, Cerebral spinal fluid, Urine, Surgical wound	General ICU and ICU II, Thoracic and Coronary Surgery units, General Care, Cardiology, Nephrology, Nursery, Hematology, Orthopedy, and Infectious Diseases
Ia	MDR	2011	02	-	01	Intravenous catheter	Pneumology
Ib	MDR	2012	01	-	01	Intravenous catheter	General Care
II	MDR	2009, 2012	03	02	01	Tracheal aspirate, Blood	General ICU, Thoracic Surgery, Infectious Diseases
III	Non-MDR	2009	01	01	-	Urine	Infectious Diseases
IV	Non-MDR	2009	01	01	-	Surgical wound	Thoracic Surgery
V	MDR	2010	01	-	01	Blood	Dermatology
VI	MDR	2011, 2012	03	-	03	Blood	General and Cardiac ICUs
VIa	MDR	2012	01	-	01	Intravenous catheter	General Care
VII	MDR	2011	01	01	-	Intravenous catheter	Pediatrics
VIII	MDR	2010	01	-	01	Intravenous catheter	Infectious Diseases
IX	MDR	2012	02	-	02	Blood, Intravenous catheter	Hematology, Pediatrics
X	Non-MDR	2012	01	-	01	Intravenous catheter	Neonatal ICU

Legend: PFGE: Pulsed-field gel electrophoresis; ICU: Intensive care Unit; MDR: Multidrug-resistance.[1,21]
Source: The authors (2021).

Diversity of clinical sites and correspondent frequency of *C. striatum* strains

Since *C. striatum* infections were not restricted to ICUs, but had also emerged in surgery units, dispersion throughout hospital wards was considered a matter of concern. Epidemiological and clinical-microbiological features of (130) *C. striatum* strains isolated from infected patients attended at HUPE/UERJ analyzed in the present study were displayed in Figure 2B and Table 2. Remarkably, *C. striatum* pathogen was present right from the beginning until the end of the study period, occurring in over twenty distinct wards, and fifteen different clinical specimens. Data showed that *C. striatum* strains were found as the etiologic agent of nosocomial infections in different clinical sites, predominantly of lower respiratory tract and bloodstream of several patients: tracheal aspirate; bronchoalveolar lavage and sputum; blood samples and intravenous catheters; urine; surgical wounds; cerebrospinal fluid; peritoneal fluid; bone fragment; hallux tendon, skin ulcer; nasopharynx/oropharynx swabs; eye secretion.

Table 2. Epidemiological and clinical-microbiological features of *Corynebacterium striatum strains* (n=130) isolated from infected patients attended at Hospital Universitário Pedro Ernesto (HUPE/UERJ), located at Rio de Janeiro city, Brazil, during a nine-year period (August 2009 - August 2018)

Clinical samples/ Hospital wards	Number of strains in wards	Year (number of strains) and Antimicrobial resistance profiles	
Tracheal aspirate		Total =53	MDR (n=53)
Intensive Care Units-ICUs	13	2009 (n=03) */pa and pb ; 2010 (n=02)*; 2011 (n=02); 2012 (n=01); 2013 (n=01); 2014(n=01); 2015 (n=01); 2016 (n=01); 2018 (n=01)	
Thoracic Surgery	04	2009 (n=01) */#; 2010 (n=01) *; 2011 (n=01); 2014 (n=01)	
Nursery units	07	2009 (n=01) *; 2010 (n=01); 2011 (n=03); 2015 (n=01) ##; 2018 (n=01)	
Nephrology	02	2009 (n=01) *; 2012 (n=01)	
General Care	16	2010 (n=01); 2011 (n=03); 2012 (n=07); 2013 (n=02); 2014 (n=03)	
Pediatric	03	2011 (n=01); 2018 (n=02)	
Coronary Care	02	2012 (n=02)	
Infectious Diseases	02	2013 (n=02)	
Pneumology / Cystic fibrosis	04	2014 (n=01); 2015 (n=01); 2016 (n=01/01##)	
NI	01	2011 (n=01)	
Additional data: General (n=10), Cardiac (n=01); ICU II (n=01); PFGE-types I, and II; Van - R (n=02); Lzd - R (n=04); Registered fatal cases (n=04); Outpatient (n=02) ## + K pneumoniae(n=01) #;'			
Bronchoalveolar lavage -BAL/ Sputum		Total = 04 (02/02)	MDR (n=04)
Nursery units	02	2009 (n=01) */ #; 2012 (n=01)	
Nephrology	01	2012 (n=01)	
General Care		2012 (n=01)	
Additional data: PFGE-type I; BAL-Registered fatal case#;			
Blood		Total = 21	MDR (n=19) /non- MDRS (n=02)
General ICU	05	2009 (n=01) *; 2011 (n=03); 2013 (n=01) S	
Thoracic Surgery	01	2012 (n=01)	
Cardiac ICU	04	2011 (n=01); 2013 (n=02); 2014 (n=01)	
General Care	01	2012 (n=01)	
Nursery units	02	2011 (n=01); 2012 (n=01)	
Infectious Diseases	03	2009 (n=02) */pb ## ; 2010 (n=01)	
Hematology	01	2011 (n=01) #	
Orthopedic	01	2011 (n=01) #	
Dermatology	01	2010 (n=01)	
Pediatric	01	2012 (n=01)	
Adolescent		2014 (n=01) S	
Additional data: PFGE-types I, II, V, VI, IX; Van- R (n=01); Registered fatal cases# (n=02); + CNS (n=01) ##			
Central venous catheter		Total =13	MDR (n=12) / non-MDRS (n=01)
General ICU	03	2011 (n=02); 2012 (n=01)	
General Care	04	2012 (n=03); 2013 (n=01)	
Neonatal ICU	01	2012 (n=01) S	
HMJ - Pediatric	01	2011 (n=01)	
Infectious Diseases	01	2010 (n=01)	
Pneumology	01	2011 (n=01) #	

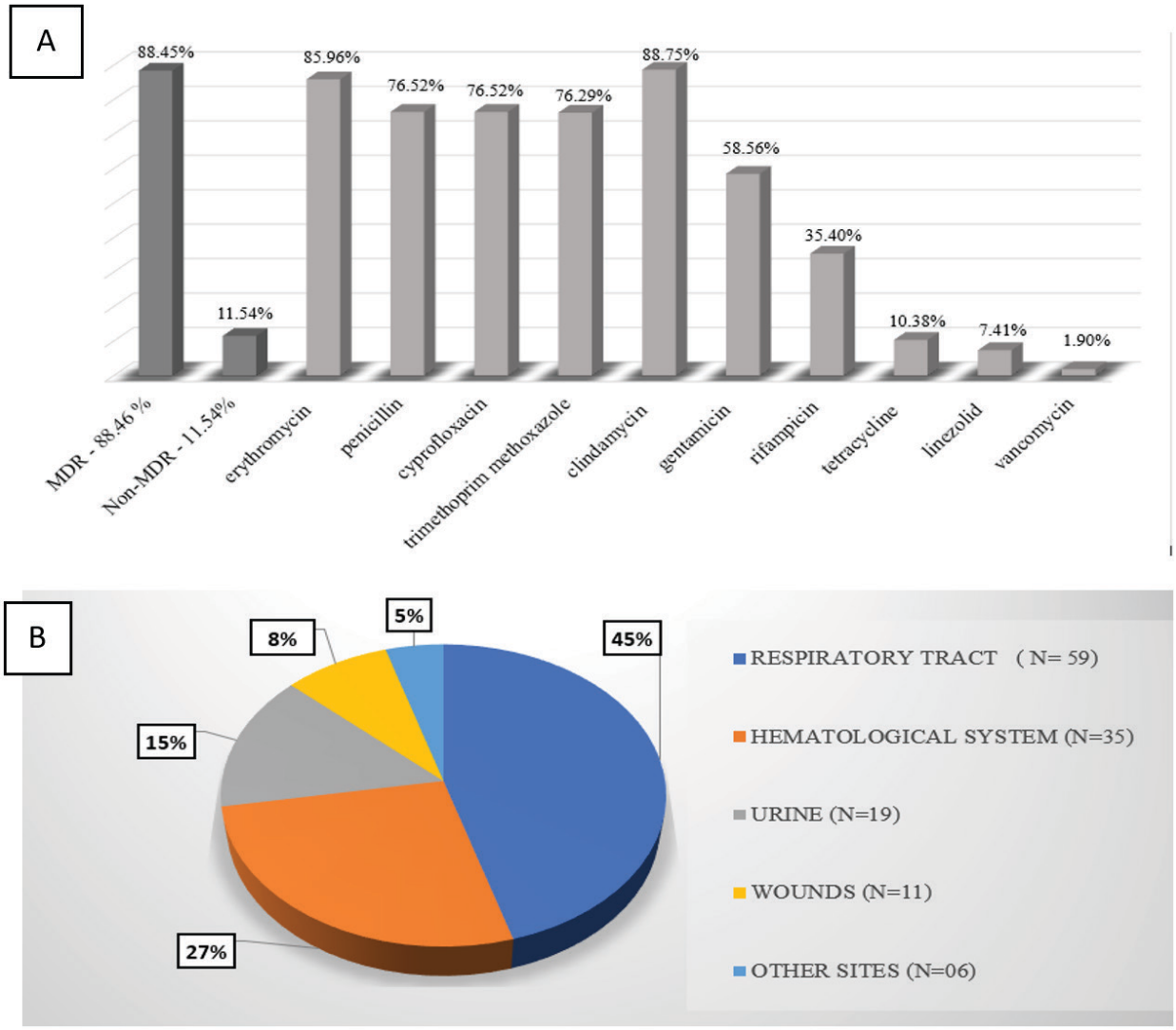
Table 2 (cont.). Epidemiological and clinical-microbiological features of *Corynebacterium striatum strains* (n=130) isolated from infected patients attended at Hospital Universitário Pedro Ernesto (HUPE/UERJ), located at Rio de Janeiro city, Brazil, during a nine-year period (August 2009 - August 2018)

Clinical samples/ Hospital wards	Number of strains in wards	Year (number of strains) and Antimicrobial resistance profiles	
Neurology	01	2012 (n=01)	
Cardiology		2012 (n=01)	
Additional data: Catheter-tips (n=06); PFGE-types I, Ia, Ib, VIa, VII, VIII and XS; + CNS (n=01) #01			
Urine		Total = 19	MDR (n=12) / non-MDR S (n=07)
General ICU	01	2009 (n=01) *	
Nephrology	12	2010 (n=01); 2011 (n=05) S; 2012 (n=03) S; 2015 (n=02); 2013 (n=01) S	
Thoracic Surgery	01	2013 (n=01)	
General Care	01	2013 (n=01) #	
Infectious Diseases	01	2009 (n=01) * S	
PCP - Clinic	01	2013 (n=01)	
NI	02	2011 (n=02) S	
Additional data: PFGE-types I and III S; Lzd-R (n=02); Nephrology ward - Renal transplant (n= 10) hospitalized and/or outpatients (12); + CNS/ K pneumoniae (n=01) #02			
Surgical wound		Total = 07	MDR (n=05) /non-MDR S (n=02)
Thoracic Surgery	02	2009 (n=01) */ S; 2015 (n=01)	
General ICU	01	2013 (n=01)	
Nursery	01	2011 (n=01)	
Infectious Diseases	01	2013 (n=01) S	
Pediatric	02	2011 (n=01); 2014 (n=01)	
Additional data: PFGE-types I, and IV S; Lzd-R (n=01)			
Cerebrospinal fluid		Total = 01	MDR (n=01)
ICU II	01	2010 (n=01) */pa #	
PFGE-type I; Registered fatal case #01			
Peritoneal fluid		Total = 02	MDR (n=02)
General ICU	01	2012 (n=01)	
Gastroenterology	01	2012 (n=01) ##	
Additional data: Outpatient ##			
Bone / Tendon material		Total = 02	MDR (n=01) / non-MDR S (n=01)
Vascular	01	2012 (n=01)	
NI	01	2011 (n=01) S	
Additional data: Hallux tendon S			
Skin ulcer		Total = 01	MDR (n=01)
Dermatology	01	2013 (n=01)	
Nasopharynx / Oropharynx swabs		Total = 02	(01/01) MDR (n=02)
Psychiatric	01	2011 (n=01) ##	
General ICU	01	2013 (n=01)	
Additional data: Outpatient ##			
Eye secretion		Total = 01	MDR (n=01)
Vascular	01	2012 (n=01)	

*, Nosocomial outbreak period, (n=15); HMJ, Hospital Menino Jesus/HUPE ward; PCP, Piquet-Carneiro Policlinic/ HUPE ward; pa and pb, two C. striatum strains isolated from one patient a and patient b; MDR, Multidrug resistance-profile; S, non-multidrug resistant strains; Van-R, Vancomycin-Resistance; Lzd-R, Linezolid-Resistance.

Source: The authors (2021).

Figure 2. *Corynebacterium striatum* **strains (%) expressing heterogenic multidrug resistance and non-multidrug resistance profiles identified as Brazilian nosocomial pathogens (A); Diversity and frequency of** *C. striatum* **strains isolated from clinical specimens of infected patients attended at tertiary care hospital (HUPE/UERJ) located in Rio de Janeiro metropolitan area during August 2009 to August 2018 (B)**



Source: The authors (2021).

During the nosocomial outbreak period (2009-April 2010), MDR *C. striatum* strains expressing PFGE-type I profiles were most frequently (78.57%) observed, especially among inpatients admitted to the ICUs and surgical wards (n = 10). *C. striatum* infections were observed in the General ICU (n = 4), cardiac ICU (n = 1), ICU II (n = 2), Thoracic surgery (n = 3), Nursery 18 (n = 2), Infectious Diseases (n = 2) and Urology (n = 1) wards. *C. striatum* nosocomial pathogens isolated from tracheal aspirate specimens obtained from patients undergoing endotracheal intubation or pneumonia were mostly of PFGE-type I. Later, MDR *C. striatum* presenting PFGE-type I were also isolated from infected patients of other hospital wards: Hematology, Orthopedic and Infectious Diseases, including patients with bloodstream and/or catheter-related infections. An increased number of bloodstream and catheter-related nosocomial in-

fections caused by *C. striatum* strains of varied PFGE-types was verified, mostly expressing MDR profiles: PFGE-types I, II, V, VI, IX; PFGE-types I, Ia, Ib, VIa, VII and VIII X, respectively. PFGE profile X was related to a case of catheter-related infection in a three-month-old patient from Neonatal ICU. Non-MDR *C. striatum* strains of two distinct PFGE-profiles types III, and IV strains were isolated from urine (PFGE-type III), and surgical wound (PFGE-type III) of two patients attended at Infectious Diseases unit.

Current data reinforced the endemic condition of *C. striatum* as a nosocomial pathogen and dissemination among hospital wards of *C. striatum* strains expressing different antimicrobial susceptibility profiles and isolated from different clinical sites of infection of both hospitalized and outpatients. Interestingly, *C. striatum* strains were verified as pathogens of kidney transplant recipients (n=10) included at-risk populations, and predominantly isolated from urine samples of outpatients (n=09). Moreover, a total of *C. striatum* strains (n=19) expressing MDR (n=12) and non-MDR (n=07) profiles were isolated from urine samples of infected patients from other HUPE-wards in addition to Nephrology: General ICU, Thoracic Surgery, General Care, Infectious Diseases and PCP-Clinic. Additionally, most of *C. striatum* strains expressing MDR (n=08) profiles were isolated from urine samples of non-hospitalized infected patients attended at Nephrology ward while non-MDR *C. striatum* strains were also associated with nosocomial infection of urinary tract origin in some other wards.

Current analyses of pediatric data from the Brazilian tertiary teaching hospital demonstrated that nine *C. striatum* clinical strains were isolated from infected children of different ages, including one adolescent patient: blood (n=02), intravenous catheter (n=02), tracheal aspirate (n=03), and surgical wound (n=02). Most of the cases in the Pediatric unit (n=07) had been associated with invasive devices (n=05). Bloodstream infections making use or not of intravenous catheter devices were the main site of infection (n=04), and *C. striatum* strains expressing MDR profiles were isolated from pediatric samples in most cases (n=07). *C. striatum* strain expressing non-MDR profile was also isolated from blood samples of an adolescent patient. Moreover, a non-MDR *C. striatum* strain of PFGE-type X isolated from the intravenous catheter of a three-month-old patient from Neonatal ICU.

During the period of study, outcomes of patients were partially informed and fatal cases mostly occurred when *C. striatum* strains were grown in pure culture and from tracheal aspirates of hospitalized patients. In two opportunities, MDR *C. striatum* strains were isolated from distinct clinical sites or samples of patients with severe invasive diseases and fatal outcome: (a) tracheal aspirate and blood samples (PFGE-Type I strains / ICU II); (b) two blood samples (PFGE-Type II strains/Infectious Diseases ward). In January 2016, a case of an infected patient with a MDR *C. striatum* strain isolated from tracheal aspirate of attended at Cystic fibrosis ward with fatal outcome was verified.

Discussion

Hospital-acquired infections are responsible for significantly higher mortality rates, length of stay and hospital costs, being an increasing cause for concern in healthcare worldwide.[26,27] *C. striatum* have been increasingly certified as a potentially pathogenic microorganism during the last decades. During a 44-year period, over 218 studies related to C. *striatum* human infections and nosocomial outbreaks were reported.[19] Nevertheless, routine procedures for laboratorial identification of *Corynebacterium* spp. remain uncommonly undertaken in many countries or IGPR clinical isolates are frequently considered as contaminants and/or underestimated by health professionals. [1,5,6,28]

In South America, reported cases of *C. striatum* infections and nosocomial outbreaks remain scarce and have been mostly reported in Brazil. Therefore, continuous survey of pathogenic *Corynebacterium* spp., in tropical and developing countries is essential, as already done for decades in the Brazilian LDCIC/FCM/UERJ laboratory.[1,5-8,19,21,23]

In Brazil, a low number of *C. striatum* strains isolated from representative clinical sites of hospitalized infected patients were formerly reported.[5,6,29] Interestingly, *C. striatum* strains expressing MDR profiles were isolated among wound/abscess (n=04) and urine (n=04) samples from infected patients attended at HUPE/UERJ from September 1993 to December 1998.[5] Eleven years later (2009-April 2010), the nosocomial outbreak was reported and fifteen *C. striatum* strains, mainly expressing MDR profiles, were isolated from infected patients of five different hospital wards, mostly from patients admitted to ICUs and surgical units.[1] The present investigation regarding nosocomial infections caused by *C. striatum* pathogen verified the endemicity status and the extensively dissemina-

tion among wards of 130 *C. striatum* strains, mostly expressing MDR profiles, during a nine-year period (2009-2018) in HUPE/UERJ, a Brazilian tertiary-care hospital located at Rio de Janeiro metropolitan area. Data showed an increase in the number of *C. striatum* clinical isolates from 2009 to 2012, with highest numbers during 2011 and 2012. During 2013, a decrease in number of *C. striatum* clinical isolates was noticed, possibly related to a reduction in number of attended patients at the hospital unit.

During the present study, MDR *C. striatum* strains were mostly isolated in pure cultures, but in some opportunities were also co-isolated with other pathogens in cultures from tracheal aspirate, blood, catheter tips or urine samples, as also verified in previous reports. Consequently, health professionals and specialists in clinical bacteriology laboratories cannot promptly discard *C. striatum* strains as contaminants, especially when isolated from chronically impaired patients and/or using invasive devices.[5,6,12,30,31]

In HUPE/UERJ, most of *C. striatum* strains expressing MDR profiles were isolated from tracheal aspirate samples of infected patients attended at ICUs, surgical and other hospital wards, including Pneumology, Cystic fibrosis, Nephrology, Pediatric and Adolescent units. Notified fatal outcomes, included a cystic fibrosis patient with MDR *C. striatum* infection. *C. striatum* strains expressing MDR profiles were mostly isolated from tracheal aspirate among hospitalized patients during post and nosocomial outbreak period, including intubated patients receiving mechanical ventilation.

Studies on nosocomial pneumonia are mainly related to the United States and European countries, whereas studies from around the world are missing.[32] In France, a recent research emphasized the fact of the clinical diagnosis of pneumonia in mechanically ventilated patients remains challenging and controversial acknowledge that despite reaching consensus, some cases may have been debatable. The study, focusing on critically ill patients showed that *Corynebacterium* spp. (n=13; 58% fatal outcome) to be responsible for pneumonia in mechanically ventilated patients attended at ICU, including *C. striatum* strains. Respiratory failure leading to acute respiratory distress syndrome, septic shock, multiorgan failure, and care withdrawal were the principal causes of ICU death. Impaired airway protection, decreased lung clearance, damaged lung structure, antibiotic exposure, primary immunocompromised conditions or acquired immunodeficiency after a prolonged stay in ICU were all conditions that can be encountered in ICU, and that may promote the onset of pneumonia caused by *Corynebacterium* spp.[33]

Nowadays, the risk of pneumonia is increased in the intubated patients receiving mechanical ventilation and the ventilator associated pneumonia nosocomial pneumonia due to mechanical ventilation procedures in mechanically ventilated patients is a matter of concern world wild, including Brazil.[34] Remarkably, current findings also indicated ventilator support as a risk factor for acquiring *C. striatum* infection. Therefore, *C. striatum* strains, especially when expressing MDR profiles, should be considered as clinically relevant when isolated in pure culture, and/or predominantly from patients with hospital acquired infections in the respiratory tract and/or ventilator-associated pneumonia, as previously reported for *Corynebacterium* spp. and other human pathogens.[12,29,33,32,35]

Moreover, MDR *C. striatum* strains were presently isolated from sputum of infected patients, as previously reported in other studies. Data from Canada verified *C. striatum* as the most frequent pathogen (26.2%) among non-diphtheriae *Corynebacterium* spp, and predominantly isolated from sputum (26%) and blood (14%) samples.[21] In Spain, a nosocomial outbreak due to *C. striatum* infection in patients with Chronic Obstructive Pulmonary Disease occurred with transmission from patients and via caretakers of patients. A total of 21 strains were isolated from sputum of infected patients during an 18 month-period.[4] In the Brazilian Southern region, a fatal case of multiple pulmonary nodules caused by a MDR *C. striatum* in an elderly immunocompetent patient was reported. *C. striatum* strain was isolated in pure culture from lung fragment of patient, but data of culture from sputum samples were not described.[29] Accordingly, analysis of the presence of *Corynebacterium* spp, in laboratorial cultures from sputum of patient is a relevant procedure that may also anticipate the diagnosis of severe infections in the respiratory tract, sometimes avoiding or postponing harmful invasive procedures for collecting clinical samples.

During the nine-year period of study in HUPE/UERJ, *C. striatum* pathogen was also isolated from a high number of blood (n=21) and intravenous-catheter samples (n=13) from patients with hematogenic infections, including a case of systemic infection caused by MDR *C. striatum* with fatal outcome. In a previous reported

study, MDR *C. striatum* strains isolated from patients presenting bloodstream (n=13) and catheter-related (n=10) nosocomial infections attended at HUPE/UERJ were mostly isolated in pure cultures (n=18) or in significant numbers (n=05).[21]

Since *C. striatum* is a potentially pathogenic species commonly found in human skin and nasal microbiota, the ability of colonization of unharmed epithelial surfaces may contribute to an increased risk of invasive infections, as previously described for *Staphylococcus aureus*.[6,12] In Japan, cases of nosocomial-acquired hematogenic infections due to MDR *C. striatum* strains (n=24) were confirmed in patients of adult age groups with underlying disease submitted to long hospitalization period. Most of patients with *C. striatum* bacteremia were using invasive medical devices, such as a central venous catheter.[36] In Sweden, cases of community-acquired bacteremia due to *C. striatum* strains (n=08) in older males with comorbidities were recently investigated. *C. striatum* caused infective endocarditis were diagnosed in two patients with heart valve prosthesis, one with fatal outcome.[37]

In accordance with previous studies, current data emphasized that *C. striatum* strains, especially when expressing MDR profiles, isolated in pure culture or in significant number from blood and catheter segments, including catheter insertion sites, should be recognized as a true pathogen with ability of causing invasive infection rather than dismissed as a contamination from normal skin flora.[21]

Interestingly, MDR and non-MDR *C. striatum* strains were firstly demonstrated as etiologic agents of urinary tract infections from hospitalized kidney transplant recipients, mostly outpatients, attended at the Nephrology ward of HUPE/UERJ. In a previous research conducted at the Republic of Korea, MDR *C. striatum* strains were mostly recovered from urine samples of patients (35.8%), diagnosed with infections mainly characterized as nosocomial and community-acquired diseases. Comorbidities included cardiovascular, malignancy, renal, and transplantation.[30] Recently, a review of literature demonstrated the occurrence of individuals with end-stage kidney disease - ESKD on hemodialysis who developed *C. striatum* bacteremia.[31] In the Brazilian Cancer Reference Center – INCA/RJ, cases of infections in patients with neoplastic disease caused by MDR *C. striatum* strains in the respiratory tract (n=04) and surgical wounds (n=02) were also reported.[6] A case of infection by a non-MDR *C. striatum* strain in a malignant cutaneous lesion from a 27-year-old male patient was also reported in South region of Brazil.[38]

Moreover, a MDR *C. striatum* strain was currently isolated from eye secretion of an infected inpatient attended at the Vascular ward. *Corynebacterium* spp. were observed in the microbiome of the ocular surface from healthy adults. Over ten potentially pathogenic species, including *C. striatum*, have been reported as etiologic agents of severe and mild ocular infections.[35,39]

In HUPE/UERJ, most of *C. striatum* strains were obtained from tracheal aspirates (n=52), blood s (n=21), intravenous catheters (n=13), urine (n=19) and surgical wounds (n=07), among other clinical samples of infected patients. Higher virulence potential to human hosts was observed for some MDR and non-MDR *C. striatum* strains related to cases of severe illness and even death of patients, and survival against environmental stress conditions. Therefore, data reinforced the virulence potential of *C. striatum* strains expressing MDR profiles and non-MDR profiles within human hosts and increased mechanisms of protection and survival against environmental stress conditions, including resistance to antimicrobial agents used in therapy, antiseptics and disinfectants used in nosocomial environment.

During the last decades, the ability of biofilm formation has been increasingly recognized as an essential mechanism involved in the pathogenic potential of human causative agents of nosocomial and community-acquired infections associated (or not) with the use of medical devices. Biofilm formation has been shown to enhance virulence potential by contributing to bacterial adherence to abiotic and biotic surfaces, metabolite exchange, cellular communication, and protection of varied Gram-positive and Gram-negative pathogens against host immune defenses and antimicrobial agents.[36] Earlier studies also verified the ability of biofilm formation by diverse pathogenic *Corynebacterium* species, including *C. striatum*.[23,37-40]

Souza and co-workers demonstrated biofilm production by *C. striatum* of PFGE profiles I to IV associated with nosocomial outbreak in HUPE/UERJ on hydrophilic and hydrophobic abiotic surfaces, at different levels. The highest ability of biofilm formation was expressed by a MDR *C. striatum* strain representative of the predominantly PFGE-type I isolated during the nosocomial outbreak. Lately, MDR

C. striatum PFGE-type I clinical isolates from patients undergoing endotracheal intubation procedures as well as PFGE-type I strains were characterized as etiologic agents of bloodstream and catheter-related infections also exhibiting high ability of adherence, survival, and production of mature biofilms on catheter segments (polyurethane and/or silicone) and metal (steel) surfaces.[21,23]

In 2009, reported cases of *C. striatum* nosocomial infections were mostly associated with use of invasive medical devices, not only tubes or catheters, but also surgical wound wires. Interestingly, all *C. striatum* clinical isolates were described by that time in Italy, as vancomycin-resistant.[35] During the reported nosocomial outbreak in Spain during 2004 and 2005, MDR *C. striatum* strains were also found 89% vancomycin-resistant.[4,18] In Brazil, most (87%) of MDR *C. striatum* strains PFGE-types I and II were identified as susceptible to tetracycline, linezolid, and vancomycin during the HUPE/UERJ outbreak. Moreover, 76.2% of MDR *C. striatum* strains, independent of PFGE profiles, isolated from patients with bloodstream and catheter-related infections and 100% susceptible linezolid and vancomycin, daptomycin.[1,21] In the present nine-year period of study, demonstrated that most endemic *C. striatum* nosocomial isolates presented high resistance levels (≥ 80 %) to antimicrobials agents frequently used to treat Gram-positive infections, especially clindamycin, erythromycin, ciprofloxacin and penicillin. Worryingly, emergence of vancomycin-resistance and linezolid-resistance was observed among *C. striatum* strains expressing MDR profiles isolated from clinical isolates of infected patients attended in HUPE/UERJ.

Conclusion

Cases of nosocomial infections and outbreaks due to *C. striatum* pathogen, as well as endemicity and dissemination in hospital environments must remain a matter of concern among researchers, epidemiologists and the medical community in South America and other continents. Nowadays, health professionals must not promptly discard *C. striatum* strains as contaminants even when found associated with one or more potentially pathogenic strains in a clinical sample from hospitalized and outpatients, independently of age, gender, and comorbidities.

Nosocomial infections and outbreaks of MDR *C striatum* can have their origin in a single reservoir or in multiple contaminated sites. Patients may possibly acquire *C. striatum* from an environmental source or from other patients, as also reported for other pathogens. *C. striatum* strains of varied genetic types, expressing MDR and non MDR profiles were found endemic in nosocomial units and disseminated among infected patients of a wide-ranging hospital wards. Varied genetic types of MDR *C. striatum* strains were also isolated from nosocomial bloodstream and catheter-related infections in HUPE/UERJ. However, a common source and the mode of transmission could not be currently determined. Additional studies must be also conducted in this area to define the clonal nature and dissemination of MDR *C. striatum* pathogenic strains in hospital environment units in Brazil and other countries.

Strategies to control nosocomial infections caused by *C. striatum* strains, including analysis of virulence mechanism of multi-factorial nature involved in the ability to survive against host immune defenses, nosocomial stress conditions, as well as intrinsic and/or acquired mechanisms involved in antimicrobial resistance, including genes encoding resistance for varied antimicrobial agents will be further investigated.[9,21]

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Declarations:

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Original article

Effect of whole body vibration on flexibility in stroke patients: A pilot study

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Abstract

Background: The motor consequences of a stroke are mainly treated with physiotherapy and blood thinning drugs. In exercise therapy with whole body vibration, studies with other patient groups show positive effects already after a single application. In stroke patients, the effectiveness of whole body vibration is still quite inconsistent. Therefore, the present study aims to investigate the effectiveness of whole body vibration on flexibility in stroke patients. Hypothesis: Whole body vibration has a positive effect on flexibility in stroke patients. Methods: 13 stroke patients (age 68.23 ± 8.93 years, mean time past since stroke 10.82 ± 8.83 months) were randomized in two groups subjected to whole body vibration at 6 and 12 Hz, respectively. Before and after the treatment of 5 x 60 seconds with a break of 60 seconds between each set, the Sit and Reach test was performed (3 runs each, the respective mean value was evaluated). Results: Both groups improved their performance highly significantly from pre- to posttest ($F(1,11) = 9.05$; $p = 0.01$). There is no difference between groups and no interaction effect for factor time*group. Conclusions: Even lower application frequencies (6 and 12 Hz) can have a positive effect on the flexibility of stroke patients. Nevertheless, further studies must try to develop an optimal training protocol for this patient group.

Keywords: Whole body vibration (WBV); Stroke; Flexibility.

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Introduction

Rehabilitation after stroke is a long process in which patients with disabilities as a result of stroke have to relearn their activities of daily living. It is important that patients are accompanied in this process to regain their condition, cope with their disabilities and avoid further complications.[1] Typical disabilities after stroke include muscle weakness, abnormal muscle stress, or dystonia, which limit daily life.[2]

WBV has been increasingly used as a low-impact treatment method for stroke patients in recent years, which is evident from several reviews and meta-analyses.[3,4] There are virtually no side effects, only contraindications are reported. Thus, WBV application should be avoided in case of pregnancy, acute thrombosis, serious cardiovascular disease, pacemaker, recent wounds from an accident or surgery, hip and knee implants, acute hernia, discopathy, spondylolysis, severe diabetes, epilepsy, recent infections, severe migraine, tumors, recently placed intrauterine devices, metal pins or plates, kidney stones, organ failure.[5,6] However, the situation still seems to be quite inconsistent in terms of efficacy. It still does not seem to be clear, what frequency of application is best, what training frequency per week, and over how many weeks should WBV be applied. Lu et al[4] suggest that WBV does not significantly affect strength, balance, and gait performance. Park et al[3] state in their analysis that the effect of WBV on spasticity is most effective compared to all other areas investigated. However, only two studies are included here that reviewed a single application. Similarly, training times varied from 12 to 45 minutes per session. All studies were conducted with an application frequency of 20 or 30 Hz, whereby only weak to medium effect strengths were determined throughout. Nevertheless, these results should be considered positive, as they provide suggestions on how training protocols could be structured. For example, it can be seen that the effect is significantly higher for a single session than for multiple sessions; one can surmise that as the number of sessions increases and the number of training weeks increases, the effect decreases. Thus, it should be assumed that a single session per month, for example, is sufficient. Especially the results of Hanif et al[7] that show a reduction of systolic blood pressure after WBV are encouraging: Lowering blood pressure could prevent a further stroke. The results by Chan et al[8] are relevant too before the present study, because there it was shown that WBV can reduce spasticity, which should consequently have a positive effect on the patients' flexibility. The results by Tamini et al[9] show a positive effect on flexibility in obesity patients. Flexibility appears to be important in everyday life for maintaining indepen-

dence. Only with sufficient flexibility the patient is able to put on and take off his clothes and shoes by himself, to take a shower or to comb his hair.

Therefore, the present study aims to find out whether lower application frequencies and a shorter application time can also produce a positive effect on flexibility with a single application.

Hypothesis

There is a difference in performance between the application of WBV of 6 Hz and 12 Hz in flexibility, measured by the Sit and Reach test in stroke patients.

Methodology

The study was approved by the ethics committee of Saarland University, application number 16-12. Trial registration was performed at Deutsches Register Klinischer Studien, registration number DRKS00012265. The recommendations of the reporting guidelines by Wuestefeld et al.[10] are followed.

Sample of persons

The test persons were recruited via medical practices, clinics, rehabilitation facilities and self-help groups in Saarland and Rhineland-Palatinate (Germany). Persons with the contraindications already described (e. g. fresh bone fracture/joint replacement, severe coronary heart disease, untreated hypertension etc.) were not included according to the recommendations.[5,6] The study was conducted in the gymnasiums of the respective facilities. The sample consists of 13 persons, of whom 5 female and 8 male persons. The average hip width is 31.86 ± 1.51 cm. The average age is 68.23 ± 8.93 years, the average time past since stroke is 10.82 ± 8.83 months. Table 1 shows the characteristics of the sample.

Table 1. Characteristics of the sample of persons

	Group 1	Group 2
Sex	2 female / 4 male	3 female / 4 male
Age in years (M ± SD)	70.17 ± 8.86	66.57 ± 9.34
Hip width in cm (M ± SD)	32.40 ± 1.67	31.42 ± 1.36
Time past since stroke in months (M ± SD)	8.33 ± 8.24	12.96 ± 9.37

Source: The authors (2021).

There are no significant differences between the characteristics of group 1 and group 2.

Study design

Stroke patients were each randomized assigned to an application frequency (6 Hz or 12 Hz). The allocation to the different vibration frequencies was randomized by drawing lots.

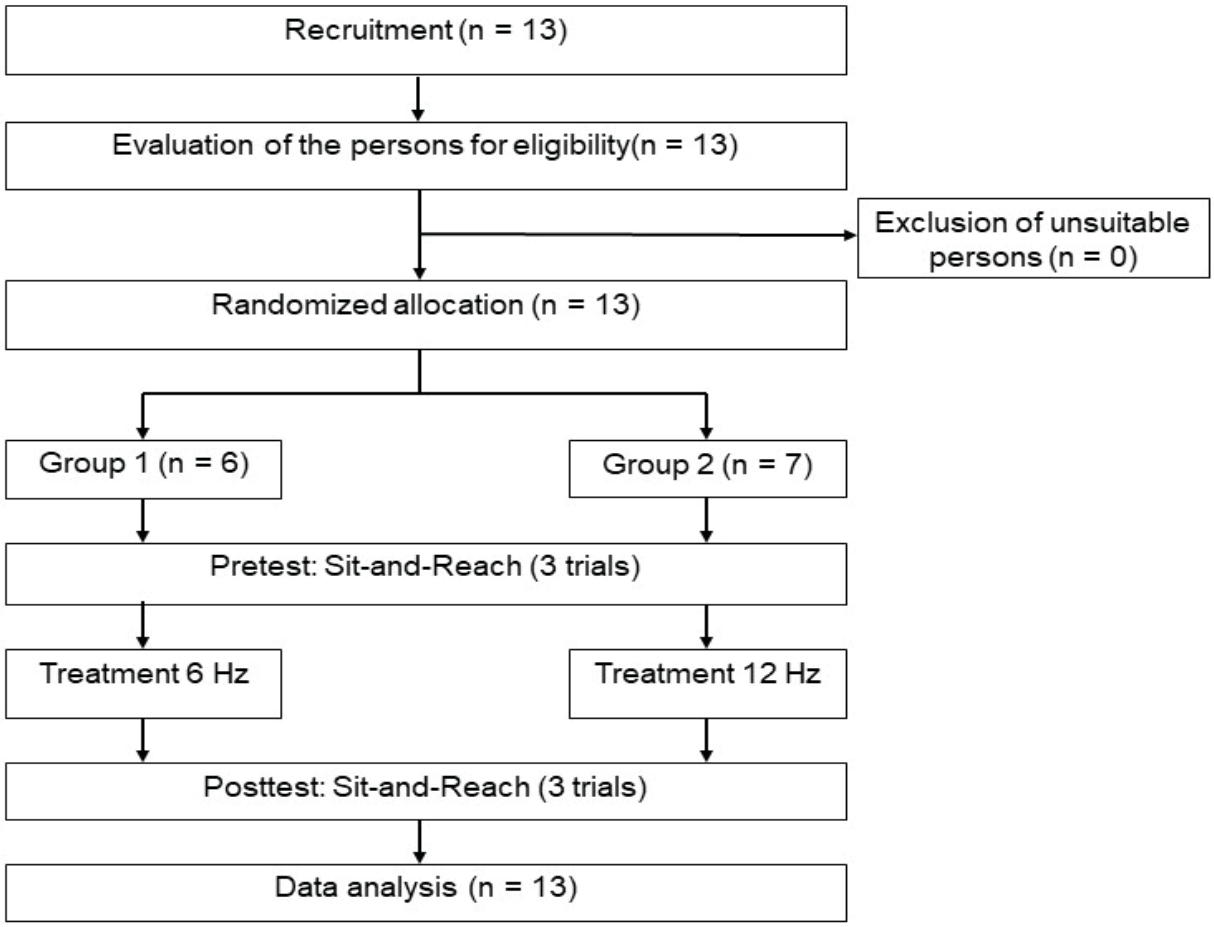
Outcome measurement

To measure the flexibility of the participants, the Sit and Reach test was performed. This procedure is an item of the Senior Fitness Test and is used to test the mobility of the hip joints and the stretching ability of the posterior thigh muscles. The test person sits with extended legs on a flat surface (floor, massage table or table) to which a measuring rod is attached. The person should push the hands towards the soles of the feet as far as possible with arms outstretched. The furthest reach of the fingertips is measured in centimeters, which can be held for two seconds without swaying.[11] The level of the sole of the foot corresponds to zero. Above the level of the sole of the foot the values are in the negative range (-1 to -30), below the level of the sole of the foot the values are in the positive range (1 to 30). As higher the value obtained, the better the flexibility. Both before and after the treatment, the person receives a trial test and then three further tests, the mean value of which is used for statistical analysis.

Intervention protocol

A side-alternating vibration platform (Galileo med Advanced) from Novotec Medical was used as treatment. Two different constant, immediately full vibration frequencies (6 Hz for group 1 and 12 Hz for group 2) with an amplitude of 3 mm were used. The test persons were instructed to stand barefoot as upright and relaxed as possible with slightly bent knees (26 to 30°) on the markers on the platform (distance between feet 31.9 cm) without holding on to the platform, as recommended.[12-14] The test persons were not informed which group they belonged to. For this reason, the display was covered. The examiner was also blinded. Five sets of 60 seconds each with a 60 second pause between the sets of static exercise (holding the stand position) with the corresponding frequency were applied, so there were 5 minutes of WBV in total for each participant. There was no muscle warmup before WBV. Figure 1 shows the course of the study.

Figure 1. Flow diagram of the study process



Source: The authors (2021).

Data analysis

SPSS Version 26 software was used. A t-test was used to compare group characteristics (age, hip width, time past since stroke) and flexibility in the pretest. Levene test was used to determine homogeneity in flexibility between groups. An ANOVA with measurement repetition was calculated. The effects time (within, pre- to posttest), group (between, 6 Hz vs. 12 Hz) and the interaction time*group were determined. For this purpose, the mean value from three test runs of the Sit & Reach was evaluated. The significance level was defined as $p < 0.05$.

Results

Levene test confirms in the pretest homogeneity of both groups in flexibility ($F = 0.50$, $p = 0.49$, n. s.)., the t-test shows no difference between the groups in the Sit & Reach pretest ($T = 0.71$, $p = 0.49$, n. s.).

Table 2 gives an overview of the results of the pre- and posttest for the Sit & Reach to compare the performance in the 6 Hz and 12 Hz group.

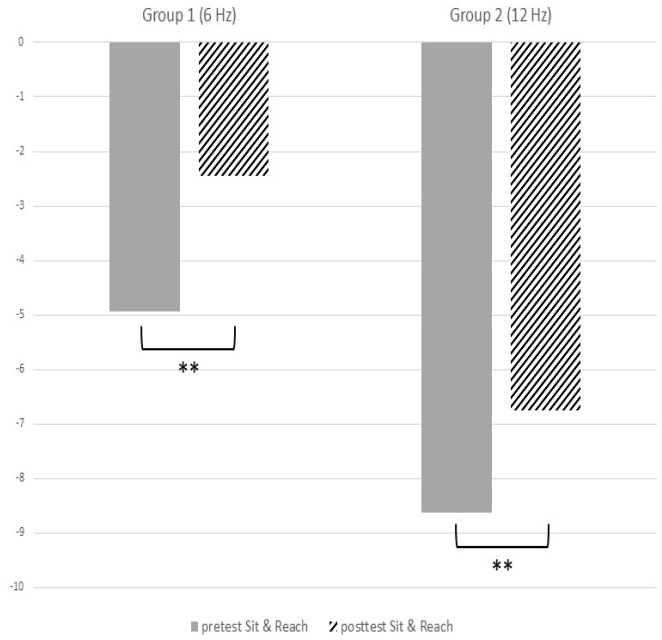
The following figure 2 shows the comparison of the results of both groups for pre-and posttest of the Sit & Reach and its significance for factor *time*.

Table 2. ANOVA Results for Sit & Reach for group 1 (6 Hz) and group 2 (12 Hz)

	Group 1 6 Hz (M ± SD)	Group 2 12 Hz (M ± SD)	Mean difference (± SE)	Time F(1,11)	Group F(1,11)	Time*group F(1,11)
Sit & Reach cm **Pretest** **Posttest**	-4.94 ± 10.43 -2.44 ± 8.43	-8.62 ± 8.31 -6.76 ± 7.89	3.67 ± 5.15 4.32 ± 4.53	9.05** (p = .01)	.69 n. s. (p = .43)	.20 n. s. (p = .67)

Source: The authors (2021).

Figure 2. Comparison between groups in Sit & Reach pre- and posttest (: p = .01)**



Source: The authors (2021).

A significant difference between pre- and posttest can be observed for both groups (p = 0.01). There is no difference between groups in pre- or posttest (p = 0.43), and no interaction effect can be found (p = 0.67).

Discussion

The aim of this present study was to investigate the effect of a single application of whole body vibration on flexibility in stroke patients. A significant change from pre- to posttest was found, but no effect for factor group and no interaction effect for factor group*time.

At first, none of the participants reported a side effect or any other negative subjective experience like dizziness or pain.

It can be assumed that the intervention may have had a positive effect on flexibility, as already shown in the study by Tamini et al.[9] although a higher vibration frequency was applied in their study. So it shows that even a lower frequency could be effective. In contrast to Chan et al,[8] a positive effect was achieved here with a lower application frequency and shorter training time. This also contradicts the assumption that low application frequencies below 20 Hz are not effective,[15] since the internal organs vibrate at a similar frequency.[16] and muscles and bones must constantly compensate for these vibrations.[17] There was no significant difference between the application frequencies so it seems that it plays no role if there are 6 Hz or 12 Hz applied.

A point that could have influenced the results is the short time past between pre- and posttest (15 min). Even if all subjects in the tests before and after each performed a test trial before the actual measurements, the muscles could have been stretched to such an extent that the effect was influenced by this as well. Vujnovich et al.[18] suggest that "the simplest explanation for the clinically observed benefits of muscle stretch is that mechanical elongation of muscle and intervening connective tissue has an effect on muscle or collagenous tissue" or that "stretching muscle tissue elicits a burst of proprioceptive activity, bombarding second- and third-order neurons located within the central nervous system". With increasing frequency of stretching, the muscle resistance decreases, i. e. the length-tension curve is shifted to the right.[19] All in all, it can be stated that a muscle reacts to a stretching stimulus in the short term with an exponential increase in torque or resistance, followed by a negatively accelerated decrease in resistance. Thus, the resistance that the muscle offers to the stretch stimulus ultimately decreases. According to findings by Scott,[20] it can be assumed that there is a short-term lengthening of the muscle and the sarcomeres.

In addition, the subject sample was very small, which may have affected the results and there was no control group. A control group could have been used to determine whether the improvement in flexibility was due to WBV alone or whether the pre-stretching in the pretest could have triggered the improvement. However, since this is only a pilot study, further investigations with larger samples should follow, also using a placebo condition.

Conclusion

It can be assumed that even lower application frequencies (6 and 12 Hz) could have a positive effect on the flexibility of stroke patients whereby the test performance of the Sit & Reach itself could have had an additional positive effect on the results. Nevertheless, further studies with larger samples and control/placebo condition must try to develop an optimal training protocol for this patient group with precise information about application frequency, number and duration of sets.

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Conflicts of interest

There are no conflicts.

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Original article

Tracking the history of circulating nucleic acids for cancer research in Brazil: A systematic review

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Abstract

Introduction: Circulating nucleic acids can be obtained by minimally invasive procedures based on liquid biopsy, which has emerged as a promising area of investigation for screening and monitoring cancer treatment. Currently, tests based on circulating nucleic acid analysis, specifically cell-free DNA (cfDNA), are commercially available for diagnostic and prognostic investigation of a number of neoplasms. Objective: To describe the research on circulating nucleic acid markers for cancer prospecting in Brazil, since this area has advanced rapidly in recent years. Methods: In this systematic review, we surveyed Brazilian publications in cancer research focused on cfDNA and cfRNA present in different fluids. Both MEDLINE-PUBMED and EMBASE databases were inspected using terms such as "circulating nucleic acids", "cancer", and "Brazil". Results: The search returned 326 articles, in which 28 Brazilian translational studies were eligible. Different methodologies were reported for different types of cancer, in which cfDNA from plasma was the most investigated biological material. Molecular investigations included quantification, somatic mutation, RNA expression, genotyping, microsatellites, blood protein interaction, and methylation. Discrepancies in the regional distribution of the studies were also observed. Conclusion: Studies on circulating nucleic acid markers have advanced significantly in the oncology field, but many others are needed to better address the clinical practice in Brazil.

Keywords: Brazil; Cancer; Circulating nucleic acids; cfDNA; cfRNA; Liquid biopsy.

Introduction

Cancer represents an important health problem worldwide. Tissue biopsies and surgical intervention are the standard strategies for the diagnosis and treatment of many cancer types. Although important, surgical interventions are very invasive procedures that may involve multiple risks. In this context, with the new technologies it is possible to track new potential cellular and genetic biomarkers that will improve this scenario, complementing the diagnosis, predicting the prognosis, and monitoring the treatment.[1] Further, since cancer is a systemic disease, these technologies can offer personalized and accurate medicine, contributing to an improved therapeutic strategy, even after surgical interventions.

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Liquid biopsy is a minimally invasive procedure that allows the screening of circulating biomarkers in the body fluids (e.g., blood, saliva, and urine), being successfully used for cancer analysis in recent years.[2] Circulating biomarkers can be molecular alterations found in circulating free or tumoral DNA and RNA (cfDNA and cfRNA), circulating tumor cells (CTCs), and extracellular vesicles (EVs); and which may be of clinical importance for cancer progression or to influence treatment.[3,4] The greater clinical interest in liquid biopsy is due to the possibility of finding great correspondence of the molecular alterations found in the circulating biomarkers reflected in the tumor tissue, thus reducing the number of invasive biopsies to monitor tumor process.

This systematic review found Brazilian publications in cancer research on circulating nucleic acids (CNAs) based on the bibliographic search from MEDLINE-PUBMED and EMBASE databases. The review focused on CNAs (cfDNA and cfRNA), since it is the most commonly studied area in liquid biopsy worldwide and approved commercial tests are available for diagnostic and prognostic investigation. As result of the search

strategy, 28 Brazilian studies on translational research met the eligibility criteria and were systematically described in this review.

Methods

Databases

The bibliographic search was performed in July 2020 in two databases: MEDLINE–PUBMED and EMBASE. The search strategy mixed generic terms, keywords, and index terms (MeSH [Medical Subject Headings] major topics, subheadings, and terms), subdivided into two synonymous term boxes (the first with terms about "circulating nucleic acids," and the second with terms about "cancer") and finally, a third restrictive search box for affiliation (Brazil OR Brasil). There were no restrictions on language and publication date. Regarding publications' variations (research articles, reviews, editorials, and abstracts), the search in the EMBASE database was restricted to research articles. Boolean operators (OR and AND) were used as connectives between searches. Lilacs and Cochrane databases and SciELO repository were consulted, but since no return from the search was obtained, they were excluded.

Inclusion and exclusion criteria

Based on the inclusion and exclusion criteria, two authors (M.C.J. and G.A.) independently selected the articles for full analysis. Review articles, editorials, abstracts, exclusive works on cell culture, trials, and articles of which the first and last authors were not Brazilian were excluded. The final two lists were compared and evaluated by a third author (L.D.).

Results

The initial search returned 326 articles, 28 of which were eligible according to the criteria selected for this study (Figure 1). To describe the articles, they were systematically reviewed and grouped by theme (Table 1). In 26/28 studies the plasma cfDNA was assessed, in 3/28 the serum cfDNA, in 2/28 the plasma RNA (1 microRNA and 1 with both microRNA and mRNA), in 1/28 cfDNA gastric wash, and in 2/28 urine cfDNA (Figure 2). Five studies included more than one fluid in their analyses. Brazilian regions were represented by São Paulo (SP), Rio de Janeiro (RJ), Alagoas (AL), and Paraná (PR). All these Brazilian studies will be presented by theme, as shown below.

cfDNA and blood protein interactions

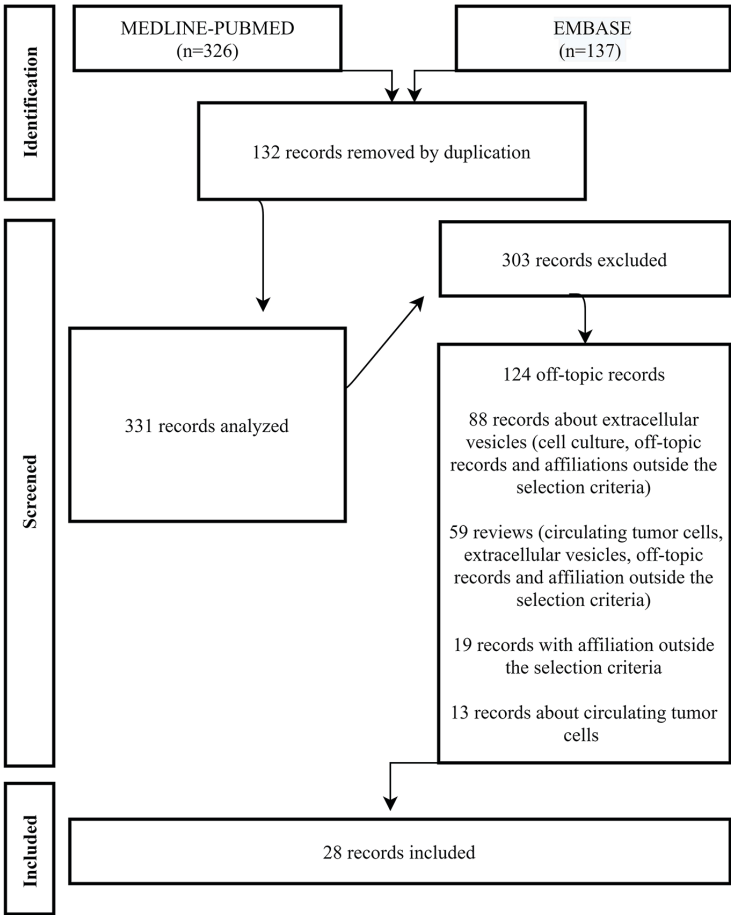
The first "made in Brazil" publication regarding cfDNA was a case report of a 65-year-old female diagnosed with small cell lung cancer in RJ.[5,6] Blood sample was collected before chemotherapy to obtain plasma cfDNA. The authors detected the formation of p53 protein-cfDNA complexes by electrophoretic-mobility shift and immunoblotting assays.

Quantification of cfDNA

Levels of CNAs in biofluids can be used to monitor minimal residual disease, relapses, and the response to therapy in oncological conditions.[7] The first Brazilian study evaluating plasma cfDNA quantity was conducted by Alves et al. in RJ using polymerase chain reaction (PCR) amplification of Long Interspersed Element-1 (LINE-1 or L1) and K562 DNA quantification standard to estimate the quantity of PCR products from glioma patients.[8] The authors suggested that the transposable elements L1 are usually released in body fluid, as they observed a greater amount of L1 PCR products in glioma patients (25 to 300μg/μL) compared to healthy controls (150μg/μL). Also in RJ, by real-time PCR-based cfDNA quantification, Machado et al. detected higher plasma cfDNA levels in pediatric patients with Burkitt lymphoma and diffuse large B-cell lymphoma at diagnosis compared to controls, and significantly decreased levels after therapy.[9]

In SP, three research groups have evaluated the cfDNA quantification in plasma from prostate cancer patients using different assays. Moreno et al. quantified plasma cfDNA using nanotechnology (Nanovue™-NV) compared with spectrophotometry (GeneQuant®).[10] The authors reported large variations values and no correlation between methods, concluding that nanotechnology was not reproducible to evaluate plasma DNA. Delgado et al. analyzed plasma cfDNA using GeneQuant® and real-time PCR at diagnosis, 3 and 6 months later.[11] The authors observed that at 3 and 6 months after diagnosis, 73.7% and 100% of the cfDNA fragments were, respectively, released from the apoptotic source (based on Alu repeat, 115-bp represented total cfDNA and 247-bp represented cfDNA from the non-apoptotic). Wroclawski et al. quantified plasma cfDNA using the PicoGreen assay and compared patients with controls (negative prostate biopsies).[12] Samples were drawn after biopsy and before treatment every 3 months during the 2-year period. The results revealed higher cfDNA concentration in patients compared to controls, and also, plasma cfDNA levels above 140 ng/mL

Figure 1. Flow diagram of the analyzed records



Authorship: The authors (2020).

Table 1. Summary of Brazilian studies on circulating nucleic acids that were included

Type of analysis	Cancer type	Biofluid	Methodology	Brazilian region (State)	References
cfDNA and blood proteins interactions	Small cell lung cancer (SCLC)	Plasma	Electrophoretic-mobility shift assay and immunoblotting	RJ	Kawamura et al., (1999, 2006)**5,6**
Quantification	Glioma	Plasma	PCR amplification	RJ	Alves et al., (2000)**8**
Quantification	Pediatric lymphoma	Plasma	Quantitative real-time PCR	RJ	Machado et al., (2010)**9**
Quantification	Prostate cancer	Plasma	Nanovue™-NV and GeneQuant	SP	Moreno et al., (2013)**10**
Quantification	Prostate cancer	Plasma	GeneQuant and quantitative real-time PCR	SP	Delgado et al., (2013)**11**
Quantification	Prostate cancer	Plasma	PicoGreen	SP	Wroclawski et al., (2013)**12**
Quantification	Colorectal cancer	Serum	Quantitative real-time PCR	AL	Filho et al., (2013)**13**
Quantification	Urothelial carcinoma of the bladder	Plasma	GeneQuant	SP	Almeida et al., (2016)**14**

Table 1 (cont.). Summary of Brazilian studies on circulating nucleic acids that were included

Type of analysis	Cancer type	Biofluid	Methodology	Brazilian region (State)	References
Quantification	Glioblastoma (GBM)	Serum	Qubit	RJ	Faria et al., (2018)**15**
Quantification	Gastric cancer	Plasma	GeneQuant	SP	Normando et al., (2018)**16**
Quantification	Bladder cancer	Plasma	Z-Scan	SP	Alves et al., (2019)**17**
Quantification	Metastatic ovarian carcinoma	Plasma	Quantitative real-time PCR	SP	Alves et al., (2020)**18**
Somatic mutations	Rectal adenocarcinoma	Plasma	ddPCR and NGS (SOLiD 4.0 platform)	SP	Carpinetti et al., (2015)**21**
Somatic mutations	Sarcoma	Plasma	ddPCR	SP	Ferreira et al., (2016)**22**
Somatic mutations	Non-small cell lung cancer (NSCLC)	Plasma	ddPCR	SP	Knebel et al., (2017)**23**
Somatic mutations	Colorectal cancer	Plasma	NGS (Ion Proton platform)	SP	Barros et al., (2018)**24**
Somatic mutations	Impalpable breast lesions	Plasma	Sanger sequencing	RJ	Delmonico et al., (2019)**25**
Somatic mutations	Colorectal cancer	Plasma	ddPCR and KRAS Screening Kit	SP	Knebel et al., (2019)**26**
Somatic mutations	Gastric adenocarcinoma	Plasma and gastric washes	NGS (Ion Proton platform)	SP	Pizzi et al., (2019)**27**
Somatic mutations	Wilms tumor	Plasma and urine	NGS (Ion Proton platform)	SP	Miguez et al., (2020)**28**
Genotyping and microsatellites assays	Head and neck squamous cell carcinomas (HNSCC)	Plasma	Automated sequencing	SP	Nunes et al., (2001)**30**
Genotyping and microsatellites assays	Breast cancer	Plasma and urine	PCR amplification	SP	Pinto et al., (2010)**31**
Genotyping and microsatellites assays	Non-small cell lung cancer (NSCLC) and Small cell lung cancer (SCLC)	Plasma and serum	PCR amplification	RJ	Cabral et al., (2010)**33**
Genotyping and microsatellites assays	Glioma	Plasma and serum	PCR amplification	RJ	Silva et al., (2013)**34**
Circulating RNA analysis	Breast cancer and breast lesions	Plasma	qRT-PCR and Artificial Neural Network (ANN)	SP	Pezuk et al., (2017)**35**
Circulating RNA analysis	Prostate cancer	Plasma	qRT-PCR	PR	Souza et al., (2017)**36**
DNA Methylation	Impalpable breast lesions	Plasma	Methylation-specific PCR	RJ	Delmonico et al., (2020)**38**

Abbreviations: AL, Alagoas; PR, Paraná; RJ, Rio de Janeiro; SP, São Paulo; PR, Paraná; ddPCR, Droplet Digital PCR; NGS, Next-Generation Sequencing.

showed a sensitivity of 66.2 % and a specificity of 87.9 % for prostate cancer diagnosis.

In AL, Filho et al. quantified the serum cfDNA by amplification of Alu repeats using quantitative real-time PCR from colorectal cancer (CRC) patients.[13] Increased levels of Alu115-qPCR and Alu247-qPCR were found in CRC non-operated patients compared to other groups, suggesting Alu-qPCR biomarkers to monitor CRC postoperative patients.

In SP, Almeida et al. quantified the plasma cfDNA levels from patients with urothelial carcinoma of the bladder using GeneQuant®.[14] Plasma was obtained before treatment and in different time points after the transurethral resection. The authors observed significant and higher mean plasma cfDNA concentrations in patients with microscopic hematuria compared to those without this condition, providing additional data for the bladder cancer prognosis.

Faria et al. compared serum cfDNA levels from patients with brain tumors eligible for intranasal delivery of perillyl alcohol (ITN-POH) with healthy controls, in RJ.[15] Samples were collected before treatment and periodically during ITN-POH, and cfDNA was quantified using Qubit®. Serum cfDNA levels from patients before ITN-POH administration were significantly higher than those found in controls. Patients who survived less than 6 months compared to those with longer survival showed higher serum cfDNA levels. Also, the ITN-POH therapy reduced cfDNA from patients, suggesting serum cfDNA to monitor brain tumor patients under ITN-POH treatment.

In SP, Normando et al. evaluated plasma cfDNA using GeneQuant® from patients diagnosed with gastric cancer.[16] Samples were collected before chemotherapy and every 3 months. The findings showed higher levels of plasma cfDNA in patients compared to controls. Also, patients who exhibited higher ctDNA levels at 3 months after chemotherapy presented a significantly lower disease-free survival (DFS), suggesting ctDNA levels to monitor disease recurrence after treatment.

Alves et al. used the Z-Scan technique to analyze cfDNA concentrations in plasma from patients diagnosed with bladder cancer, in SP, and differences in cfDNA concentrations during treatment were reported.[17]

Alves et al. analyzed the plasma ctDNA levels from patients with metastatic ovarian carcinoma, in SP.[18] Samples were drawn before treatment and afterwards each month for 6 months. Patients showing increased ctDNA from baseline after the first cycle of chemotherapy responded better to the treatment and had significant improvement in the DFS.

All of these cfDNA quantification aforementioned studies are consistent with those found in studies outside Brazil.[19,20]

Somatic mutations in cfDNA

Genetic mutations found in cfDNA can correspond to those found in the tumor tissue, thus reducing the need for invasive biopsies to monitor tumor dynamics. In SP, Carpinetti et al. analyzed the chromosomal rearrangements in the plasma cfDNA from patients with rectal adenocarcinoma.[21] Samples were obtained at diagnosis, during neoadjuvant chemoradiotherapy (nCRT), and during follow-up. cfDNA was detected using a QX200 ddPCR (Droplet Digital PCR) system and nested PCR assay. For chromosomal rearrangement assays, the DNA was sequenced in Next Generation Sequencing (NGS) platform. At least two distinct somatic rearrangements were found in the tumor samples from each patient that the authors later used to correlate with the plasma ctDNA. The authors observed that all patients showed undetectable or reduced ctDNA levels for the biomarkers after nCRT.

Also in SP, Ferreira et al. described a translocation t(11;22)(p13;q12) of the *EWS-WT1* gene fusion in plasma DNA using a QX200™ ddPCR assay from a 26-year-old male with a desmoplastic small round cell tumor (DSRCT).[22] Samples were collected periodically 3 years after surgery (4 time points) and no translocation was detected in the post-treatment, correspondent to the favorable clinical outcome.

In SP, Knebel et al. monitored *EGFR* gene mutations in plasma cfDNA from a 53-year-old woman diagnosed with non-SCLC.[23] Samples were isolated monthly after therapy (erlotinib and osimertinib) and *EGFR* mutations were evaluated by QX200™ ddPCR assays. After erlotinib progression, the authors found 3598 and 1564 copies/reaction for *EGFR*-exon19del and *EGFR*-T790M mutations, respectively. Both mutations were undetectable two weeks after osimertinib therapy. After 4 months, both mutations showed increased copies/reaction, suggesting acquired resistance (AR) to osimertinib, especially the *EGFR*-exon19del mutation.

Also in SP, Barros et al. investigated a panel of hotspot mutations in the plasma ctDNA from a 57-year-old man with metastatic CRC by NGS technology.[24] Samples were drawn before treatment and 5 times after surgery and chemotherapy. The authors detected a high frequency of *KRAS* (p.Gly12Val) and *TP53* (p.Arg175His) mutations in the first plasma sample. Both mutations frequencies were reduced 1 month after chemotherapy, but increased after the third month of treatment, showing ctDNA as a sensitive tool to monitor treatment response.

Delmonico et al. investigated the mutational profile of *PIK3CA* (exon 9 and 20), *TP53* (exon 5-8), and *CDKN2A* (exon 1, 2a, and 3) genes by Sanger sequencing in the plasma cfDNA from patients with impalpable breast lesions (BI-RADS 3 and 4), in RJ.[25] Samples were obtained before surgery and 5% and 24% of mutations were found in the cfDNA of women with benign lesions and malignant lesions, respectively. cfDNA mutations were found mostly in the *TP53* gene, especially in women exhibiting malignant lesions.

Knebel et al., in SP, evaluated *KRAS* mutations in the plasma ctDNA from a 61-year-old man with advanced CRC using QX200™ ddPCR and a *KRAS* screening commercial assay.[26] Samples were analyzed for almost 2 years and no *KRAS* mutations were found at the beginning of the cetuximab-based chemotherapy, but reaching to 33.8% about 2 months later.

Also in SP, Pizzi et al. evaluated plasma and gastric washes to assess the *TP53* mutations in the cfDNA using NGS platform from patients with gastric adenocarcinoma.[27] Samples were collected at diagnosis and after treatment. The authors found *TP53* mutations in 15.2% of gastric washes, with concordance between tumor biopsies in 82.6% of cases at diagnosis. Regarding plasma cfDNA, *TP53* mutations were found in 13% of cases, being 80.4% in concordance with biopsy samples. Post-treatment analyses revealed that only 6 cases had *TP53* mutations in tumor biopsy and 2 of them showed detectable mutation in gastric washes or plasma. Thus, the authors suggested both fluids to monitor tumor mutations and treatment response.

Miguez et al. evaluated the somatic mutations using NGS platform in the cfDNA from plasma and urine of 6 female patients with Wilms tumor in SP.[28] Before treatment, 5 patients showed at least 1 somatic mutation in tumor samples and also in body fluids, being reduced after chemotherapy.

Microsatellite assays with cfDNA

Alterations in the microsatellite's sequences are common in several kinds of cancer due to the genome instability.[29] In SP, Nunes et al. evaluated plasma cfDNA from patients with head and neck squamous cell carcinomas (HNSCC) to investigate microsatellite instability (MSI) and loss of heterozygosity (LOH).[30] Loss of heterozygosity and/or MSI were found in 64% of the tumor biopsies in at least 1 locus, in which 29% of these alterations were also detected in the plasma cfDNA, especially from those with advanced clinical stages. In SP, Pinto et al. investigated the induction of alkylating agent-based chemotherapy (ACHT) on MSI, before and after treatment, by analyzing plasma and urine cfDNA from untreated patients diagnosed with breast cancer.[31] The authors found at least 1 MSI or LOH event in 80% of both plasma and urine cfDNA samples at 6 months, especially for BAT40 and BAT26 markers, suggesting that ACHT could induce MSI.

Genotyping with cfDNA

The glutathione S-transferase (GST) gene family encodes metabolic enzymes involved in xenobiotic detoxification and therefore the GST polymorphisms may influence its activities.[32] In RJ, Cabral et al. evaluated the *GSTM1* and *GSTT1* genotypes by PCR amplification from plasma/serum cfDNA of lung cancer patients.[33] Lung cancer risk was significantly higher in tobacco smoker patients carrying *GSTM1* and *GSTT1* null genotypes. Also in RJ, Silva et al. analyzed the influence of *GSTM1* and *GSTT1* polymorphisms in plasma/serum cfDNA in response to ITN-POH administration from patients with malignant glioma.[34] Significant difference was found between patients and controls for *GSTT1* deletion. Patients with *GSTT1* null genotype had significantly lower survival rates, showing the influence of GST polymorphisms.

Circulating RNA analysis

RNA families are especially involved in gene expression process. In SP, Pezuk et al. compared plasma miRNAs from breast tumor patients (Breast Imaging-Reporting and Data System - BI-RADS 5 or 6) and controls (BI-RADS 1 or 2) using miRNome array, qRT-PCR, and the Artificial Neural Network (ANN).[35] Plasma was obtained before mammography and biopsy. As a result, the authors found miRNAs differentially represented between groups, with 46 over- and 9 underrepresented in the patients' samples. Regarding ANN, the authors found 92.46%, 87.50%, and 94.59% of accuracy, sensitivity, and specificity, respectively, suggesting this approach as a complementary method to classify BI-RADS 4 lesions. In PR, Souza et al. using *in silico* analysis compared circulating mRNAs and microRNAs from prostate cancer tissues and surrounding normal tissues (SNT) from the Cancer Genome Atlas (TCGA) database, and validated the results using the RT-qPCR assay.[36] The authors observed 2,267 genes and 49 miRNAs with differential expression between tumor and normal tissue samples. Additionally, the expression of 2 genes (OR51E2 and SIM2) and 2 cfmiRNA (miR-200c and miR-200b) were significantly associated with prostate cancer.

Methylation signature in cfDNA

Epigenetic inactivation by DNA methylation of tumor suppressor genes is associated with various neoplastic diseases due to increased genetic

instability.[37] Therefore, the methylation status in the promoter region of crucial genes should be monitored to provide additional information on cancer development and progress. Delmonico et al. investigated plasma cfDNA from women with and without impalpable breast lesions to detect the epigenetic alterations and observed that *ATM* and *p14*^{ARF} showed higher methylation rates in samples from women with malignant lesions and older than 50 years.[38] Thus, the authors described the potential detection of epigenetic changes in liquid biopsy to monitor the impalpable breast lesions.

Discussion

Liquid biopsy is a significant area of cancer studies with great prominence over the years, since it requires a minimally invasive procedure to track alterations in circulating biomarkers that potentially contribute to diagnosis, predict prognosis, and monitor response to treatment.[2,3] This systematic review described 28 Brazilian studies on circulating nucleic acids (CNAs) from cancer patients' samples with plasma and cfDNA the most investigated biological material, in which investigations included quantification (11 studies), followed by somatic mutation (8 studies), RNA expression (2 studies), genotyping, microsatellites, blood protein interaction (2 studies each one), and methylation (1 study).

Brazilian cfDNA quantification studies reported higher cfDNA levels in cancer patients compared to healthy volunteers. Decreases in cfDNA levels after treatment were also reported, being all these findings consistent with those found in studies outside Brazil.[19,20] Somatic mutations in the cfDNA samples reported in this review revealed molecular signatures that may be associated with tumor development, progression, and resistance to treatment. Additionally, Brazilian RNA-based studies reported differential expression between samples from cancer patients and healthy controls. RNAs are involved in multiple biological processes with a wide range of functions, in which dysregulations in RNA expression patterns are associated with pathological conditions. Circulating RNAs are usually found with differential expression profiles in cancer patients and may contribute to initiation and progression of oncological processes.[39]

Interesting to note, the studies were concentrated in the Southeastern region of Brazil, especially in São Paulo and Rio de Janeiro (17/28 and 9/28, respectively), followed by one study in Alagoas (Northeastern region of Brazil) and another one in Paraná (South region of the country). These regional discrepancies may reflect the differential distribution of financial resources for scientific research.

Conclusions

Liquid biopsy is a promising investigation area in cancer screening. In summary, this systematic review described 28 Brazilian studies on cfDNA and cfRNA from cancer patients' biofluids using different methodological assays, being cfDNA the most commonly analyzed CNAs. More studies on CNAs in cancer patients are needed to complement the clinical practice investigations in Brazil.

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Original article

Comparative analysis of methemoglobin, oxygen saturation and hematological parameters in smokers and non-smokers: An observational analytical cross-sectional study

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Abstract

Introduction: The formation of methemoglobin (MetHb) occurs through the oxidation of iron in hemoglobin, impairing its capacity for oxygen association and deoxygenation. On exposure to oxidizing agents, such as those present in cigarettes, this process may be more frequent, causing an increase in serum MetHb. Objectives: To evaluate and compare methemoglobin, oxygen saturation and hematological parameters between smokers and non-smokers. Materials and methods: Observational case-control study with participants classified as smokers and non-smokers, in equal number and gender. Smokers classified as moderate to very high dependence degree by the Fagerström tolerance questionnaire were included. In all subjects, oxygen saturation was assessed using portable pulse oximetry, methemoglobin levels by spectrophotometric method and hematological parameters by an automated analyzer. Parametric (Student's T-test) and non-parametric (Mann-Whitney U) tests were performed for comparison of mean values between groups. Results: There were no changes in methemoglobin rates and hematological parameters, both in relation to clinical reference values and in the statistical difference between groups. The oxygen saturation values were significantly higher in the smoking group, 96.4% versus 94.8% (p = 0.04). Conclusions: Despite the potential deleterious effects of cigarettes, in this study it was found that smoking was not a determinant of changes in methemoglobin rates and hematological parameters, when compared with non-smokers. Further studies are suggested with a robust sampling population, complementary analysis of hematological and physiological factors and verification of comorbidities, in order to elucidate a greater relationship between the presented parameters.

Keywords: Hematology; Tobacco Use Disorder; Blood Physiological Phenomena.

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Introduction

Smoking is recognized as a chronic and epidemic disease by the World Health Organization (WHO) and as one of the biggest causes of preventable early deaths in the world.[1] In Brazil, it is one of the main risk factors for death, disability or decreased productivity in people with chronic diseases, generating high costs for public health and reducing life expectancy by about six years compared to non-smokers.[1] Its relevance is due to the presence of nicotine, a psychoactive substance that generates physiological, psychological and behavioral dependence on the consumer. Burning tobacco exposes the smoker to more than 4,000 substances with different chemical properties, of which approximately 60% with carcinogenic activities and 40% recognized as toxic substances to the human body.[2,3]

The harmful effects of chronic exposure to cigarette substances are important for the onset and aggravation of pulmonary and cardiovascular diseases. The incidence of respiratory tract diseases, in general, is directly associated with smoking, such as chronic obstructive pulmonary disease (COPD) and lung cancer. While for others, the influence on the frequency, injuries on the onset, course and outcomes of the diseases is reported.[4] In smokers, the molecular, structural and functional changes in the respiratory and vascular tract are reported, such as decreased defense and cell repair functions, increased inflammatory response and decreased concentration and activity of ascorbic acid and total antioxidants.[2,5] Cell death - both by apoptosis and necrosis - induced by exposure to cigarette smoke can be explained by an increase in oxidative stress in the alveolar tissue, with its effects extending systemically through the bloodstream, affecting the blood and vascular tissues itself.[5]

Hemoglobin (Hb) is a globular protein contained within erythrocytes that is responsible for transporting blood gases, such as oxygen (O_2) and carbon dioxide (CO_2).[6,7] During this association, an electron from the iron present in the heme group can be transferred to oxygen, changing it from a ferrous (Fe^{2+}) to a ferric

(Fe^{3+}) state. In the deoxygenation process, the electron can return to the iron in heme molecule, reducing it to the ferrous state again. This iron auto-oxidation occurs naturally in healthy individuals, and the conversion of methemoglobin is carried out by the body through protective mechanisms that attempt to reverse the oxidative stress situation by reducing it to Fe^{2}+. These mechanisms occur due to physiological and enzymatic systems, such as the action of ascorbic acid, glutathione reductase (GSH), through NADH-dependent cytochrome-b5 reductase and NADPH-dependent methemoglobin reductase.[7,8]

However, about 0.5 to 3.0% of Hemoglobins do not perform this conversion, remaining in the ferric state. This prevents the formation of new reversible bonds with molecular oxygen, thus forming methemoglobin (MetHb). Therefore, the formation of MetHb is due to excessive Hb oxidation (increased production) or decreased activity of reducing enzymes. These factors can cause a decrease in circulating oxygen, and, in this situation, the hemoglobin saturation curve presents a dissociation deviation, distorting its normally sigmoid shape. In addition, MetHb decreases the cooperative release capacity of the oxygen molecule, impairing tissue oxygenation.[9,10]

Methemoglobinemia is considered as a 1.5% increase in the MetHb concentration in relation to total hemoglobin, impairing oxygen distribution. It is understood that methemoglobinemia is a possible cause of anemic hypoxia, that is, a product of the decrease in functional hemoglobin, being presented as a reduction in the oxygen transport capacity when the partial pressure of O_2 and cardiac output are regular or high. The low saturation reading of pulse oximeter (SpO_2) can support the diagnosis of methemoglobinemia in patients with central cyanosis, but the co-oximeter is a gold standard method equipment for the determination of MetHb.[9]

Methemoglobinemia can have an acute onset or can occur due to chronic exposure to methemoglobinic agents, such as drugs, pesticides, herbicides, fertilizers, industrial chemicals, nitrites and nitrates present in water and food, as well as exposure to smoke.[9,10] There is a wide variation in which such substances are able to cause methemoglobinemia, avoiding a quantitative determination in relation to exposure and the clinical condition presented. Hence, the reduction in the number of functional erythrocytes or changes in hemoglobin by chemical induction, as mentioned above, may lead to methemoglobinemia.[9,10]

Thus, the present study aimed to evaluate changes in methemoglobin, oxygen saturation and hematological clinical parameters in smoking volunteers compared to non-smoking counterparts.

Materials and methods

The present study, characterized as an analytical observational cross-sectional case-control study, was approved by the Research Ethics Committee of Universidade Paulista, Number 3.186.586/CAAE 03249518.1.0000.5512. It respected all the ethical principles described in resolution 466/12 of the Brazilian National Health Council, especially in which participation is free and spontaneous and with a confidential identity to avoid exposure. The Informed Consent Form was signed by the participants after explanation about the research objectives, being the primary and indispensable inclusion criterion in the sample.

The individuals participating in this study were recruited and invited from March to May 2019. The smoking volunteers had the inclusion criteria to be over 18 years old and to have nicotine dependence assessed through the Fagerström Tolerance Questionnaire (FTQ) translated into the Portuguese.[11] The questionnaire consists of six items, each having 2 to 4 alternatives scored to each answer. At the end, the result is obtained by the sum score, which varies from 0 to 10, indicating the level of dependence of the smoker. The reference score rank used in the questionnaire are: 0 to 2 very low; 3 to 4 low; 5 medium; 6 to 7 high; and 8 to 10 very high dependence.[11] Smoking volunteers who obtained medium to very high levels of dependence as a result were included. For the group of non-smokers, individuals over 18 years-old who reported never having smoked before were included. It was considered as an exclusion criterion, in both groups, the self-reported presence of hematological, acute or chronic respiratory diseases or other conditions that impact the blood count and methemoglobin levels, such as cancer, anemia, surgeries less than a week and chronic exposure or acute to other methemoglobinizing agents, such as those mentioned above.[9,10]

As suggested by the resolution 466/12 of the Brazilian National Health Council, as a research benefit to encourage changes to healthy attitudes, all volunteers were instructed about the harmful effects of cigarettes and the existence of the National Tobacco Control Program to stop smoking, offered at no cost by the Brazilian Ministry of Health through the National Cancer Institute.

Venous blood were collected in partnership with a private diagnostic medicine laboratory, in the facility with best access for each volunteer, with no prior preparation in relation to fasting or previously defined hour.

The laboratory has certifications that aim to guarantee the necessary quality for the analysis of this study (College of American Pathologists – CAP, Accreditation Program for Clinical Laboratories – PALC and ISO 9001).

Determination of methemoglobin assays were performed using the spectrophotometric method using a co-oximeter device, Cobas b221® (Roche Diagnostics, USA), according to the manufacturer's protocol. The technique consists of dissolving the blood in a lysing reactant and aggregating it to a high vibrational frequency hemolyser. Then the sample was loaded to an optical cuvette to measure the absorbance at seven predefined wavelengths, which allow the differentiation of the hemoglobin fractions in distinctive absorptions. With the absorbance data, the equipment itself uses matrix equations to calculate the concentrations of each fraction.

The hematological parameters observed in the red series were: Erythrocyte, Hemoglobin, Hematocrit, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC) and red cell distribution width (RDW). The assays were performed using the automated Sysmex ® analyzer device, according to the manufacturer's protocol. For RBC, Ht and RDW, the flow impedance with hydrodynamic focus methodology was used by the equipment. For Hemoglobin, colorimetry was used by the device, where all forms of hemoglobin are converted to a stable form and measured at 550nm in spectrophotometry. Finally, the MCV, MCH, MCHC parameters were obtained through calculations from the values presented above by the device itself.

The determination of oxygen saturation (SpO_2) was performed by a portable pulse oximetry device, the Nonin GO2 Achieve (Nonin Medical Inc, MN, USA), with an oxygen saturation accuracy range between 70% to 100% SpO_2.[12] The device was placed on the volunteer's index finger to measure the SpO_2 transported in the blood as a percentage of its total transport capacity, ideally above 89% SpO_2.

For data analysis, Microsoft Excel® was used for data arrangement and descriptive analysis. To verify the data distribution, the Shapiro Wilk test was used, available on the Sdittami online platform. A parametric test (Student's t test) was chosen for the comparison between two groups that had data with normal distribution, available online on the Evanmiller platform. Data in which at least one group had non-normal distribution were analyzed using the Mann-Whitney U test, available on the Socscistatistics platform. Difference of means were considered with association probabilities lower than 5% error ($p<0.05$).

Results

A total of twenty-four volunteers residing in the Federal District participated by signing the consent form. They were allocated into 2 groups according to the smoking characteristic, i.e. active smokers or non-smokers, each containing the same number of people and gender equivalence, that is, 6 men and 6 women in each smokers and non-smokers groups. For all parameters analyzed, there was no significant difference between men and women, so all comparisons were made only between the smoking characteristic. The Fagerström test for the inclusion of the twelve smoking volunteers showed a result of 58.3% for moderately dependent smoking volunteers, 25% with high dependence and 16.7% with very high dependence to nicotine.

The oxygen saturation presented in both groups was within the reference value recommended for a healthy adult individual by the Brazilian Society of Pulmonology and Phthisiology (SBPT), i.e., greater than 89%. Data distribution was non-normal and the Mann-Whitney U test was used. The mean and standard deviation of the values for the smoker group was 96.4 ± 1.1% (with a minimum value of 95% and a maximum of 98%), and for non-smokers 94.8 ± 1.8% (with a minimum value of 93% and maximum of 98%). The means between the groups of smokers and non-smokers had a significant difference ($p = 0.04$).

In the assessment of methemoglobin saturation, all values were below clinical reference, that is, less than 1.5%. From the statistical analysis it can be inferred that the mean in the smoker group was 0.72 ± 0.09% (with a minimum value of 0.50 and a maximum of 0.80%) and in the non-smoker group it was 0.72 ± 0.08% (with minimum values of 0.60 and maximum of 0.80%), with no significant difference ($p = 0.95$). Table 1 describes the oxygen saturation and methemoglobin parameters for individual and group means.

The hematological parameters under study did not show any discrepancy in relation to the clinical reference values, either individually or in the means of each group. In the statistical analysis, the parameters had a normal distribution and the Student's t-test was used to calculate the differences between the means of the groups. The exception was the MCV, which obtained free distribution and the Mann-Whitney U test was used. The results presented by the groups were compared and no significant difference was shown within the p value for each parameter (Table 2).

Table 1. Determination of oxygen saturation (SpO_2) and methemoglobin (MetHb) in a group of smoking volunteers according to the levels of dependence using the Fagerström tolerance questionnaire and non-smokers, described individually and in groups by mean ± standard deviation. Statistical significance considered for $p < 0.05$

Smokers	Smokers Dependence score	Smokers SpO2	Smokers MetHb	Non-Smokers	Non-Smokers SpO2	Non-Smokers MetHb
S-1	8	98%	0,7%	NS-1	94%	0,8%
S-2	5	97%	0,8%	NS-2	94%	0,8%
S-3	5	96%	0,5%	NS-3	96%	0,8%
S-4	5	98%	0,8%	NS-4	96%	0,6%
S-5	6	96%	0,7%	NS-5	98%	0,6%
S-6	5	96%	0,7%	NS-6	95%	0,8%
S-7	6	98%	0,7%	NS-7	95%	0,7%
S-8	5	96%	0,8%	NS-8	93%	0,7%
S-9	7	97%	0,8%	NS-9	96%	0,8%
S-10	5	95%	0,8%	NS-10	92%	0,6%
S-11	5	95%	0,8%	NS-11	92%	0,7%
S-12	8	95%	0,6%	NS-12	97%	0,8%
Mean ± standard deviation	**5,83 ± 1,19**	**96,3% ± 1,2%[a]**	**0,727 ± 0,1%[b]**		**94,8% ± 1,9%[a]**	**0,725 ± 0,09%[b]**

Legend: S = Smokers Volunteer; NS = Non-Smokers Volunteers; SpO_2 = Oxygen saturation; MetHb = methemoglobin.
Clinical reference value for $SpO_2 > 89\%$.
Clinical Reference Value for MetHb: Unexposed = 0.04 to 1.52%; Toxic level = above 15%; Lethal level = above 70%.
P-value a = 0.04; P-value b = 0.99.
Authorship: The authors (2021).

Table 2. Evaluation of the hematological parameters of the group of smokers and non-smokers, described as mean ± standard deviation. Statistical significance considered for $p < 0.05$

Hematological Parameters	Smokers (n = 12)	Non Smokers (n = 12)	p-value	Reference Values	
				Adult Men	Adult Women
Erythrocyte (/mm^3)	5,04 ± 0,2	4,90 ± 0,4	0,56	4,5 – 6,0	4,0 – 5,4
Hemoglobin (g/dL)	15,2 ± 0,8	14,4 ± 1,0	0,15	13,0 – 16,5	12,0 – 15,8
Hematocrit (%)	44,2 ± 2,2	42,8 ± 2,9	0,38	36,0 – 54,0	33,0 – 47,8
MCV (fl)	87,6 ± 3,3	87,6 ± 3,3	0,90	80,0 – 98,0	80,0 – 98,0
MCH (pg)	30,3 ± 1,3	29,4 ± 0,8	0,24	26,8 – 32,9	26,2 – 32,6
MCHC (g/dL)	34,4 ± 0,7	33,6 ± 0,4	0,05	30,0 – 36,5	30,0 – 36,5
RDW (%)	13,1 ± 0,5	13,3 ± 0,4	0,72	11,0 – 16,0	11,0 – 16,0

Legend: MCV = Mean Corpuscular Volume; MCH = Mean Corpuscular Hemoglobin; MCHC = Mean Corpuscular Hemoglobin Concentration; RDW = Red Cell Distribution Width.
Authorship: The authors (2021).

Discussion

In the present study, it was noted that even with medium to very high levels of dependence and frequent exposure to harmful agents present in cigarettes, the group of smokers did not show potential changes in methemoglobin or hemogram parameters. It was assumed that the amount of agents to which smokers are exposed is not sufficient to cause increased MetHb formation or to alter hematological parameters in the present sample.[10] For the group of non-smokers, all variables evaluated were within the clinical limits of the reference values.

Although it was not observed in the present study, others have shown that smoking caused an increase in carbon monoxide levels, being able to modify some of the hematological parameters, such as hemoglobin, leukocytes, MCV, MCH and RDW.[13,14] The increase in carbon monoxide in smokers was observed in another study in 5% of carboxyhemoglobin (COHb), based on the comparison of results obtained in smoking and non-smoking volunteers. However, no differences and changes in methemoglobin levels were observed between the groups in those studies.[13,15,16]

In this research, a statistical significance was observed in the evaluation of oxygen saturation, showing that the mean saturation in the control group, even within the reference value, was lower than the mean presented by smokers. It was considered, as observed in another study,[15] that the high saturation presented by smokers is due to the presence of carboxyhemoglobin and the limitation of the oximeter in distinguishing between oxyhemoglobin, carboxyhemoglobin, methemoglobin and other fractions of this molecule. Therefore, methemoglobin could not be considered here as interfering in the results of O_2 saturation, as it was within the reference values in both groups.[15]

The difference observed in oxygen saturation may occur in cases that different hemoglobins are not distinctable.[9] The pulse oximeter uses only two light wavelengths for hemoglobin and oxyhemoglobin, not being able to distinguish carbon monoxide from O_2, due to the amount of light absorbed being similar and falsely raising the saturation.[9] A similar case also occurs with methemoglobin, when elevated, even in small amounts, it provides erroneous estimates of oxygen saturation, delivering a higher saturation value than the real one.[17]

The difficulty in determining the direct quantitative relationship between occupational exposure to a potentially harmful agent and the likely presentation of physiological and hematological changes has been described in toxicology.[9] Although MetHb is considered an indicator of the exposure effect, it is important to note that there is a diversity of potentially methemoglobinizing agents from the tobacco combustion, which in turn also have variations related to toxicokinetics and toxicodynamics, indicating acute or chronic changes in various tissues in the human body.[2-4]

Other authors have shown different MetHb results. Despite using the same methodology for determination, the sample number used in other was higher than in this study (856 volunteers, 377 smokers and 479 non-smokers). The authors found MetHb levels in smokers was significantly higher compared to non-smokers, respectively MetHb = 0.63% and 0.56%, however, these values are still considered clinically normal.[18]

Another study aiming to verify the increase in MetHb in smokers observed the mean methemoglobin saturation of the smokers group (n=15) clinically and statistically higher compared to the non-smokers group (n=15).[19] However, the authors used manual methods to perform the determination, and in the present study the automated methodology was the one of choice, also including quality control for assurance and safety of the results.

No changes were observed in the hematological parameters evaluated in the present study. Due to exposure to carbon monoxide, hemoglobin levels could be altered, impairing oxygen bounding and an increase in the number of red blood cells as compensation.[10,20] However, the present findings corroborate other studies that obtained the absence of hematological alterations as results.[15,16,21]

The low number of individuals in each group can be pointed out as a limitation of the study, which did not allow the calculation of the statistical sampling power to be determined in a robust way. By using a larger sample, homogeneity could be assessed in relation to other factors associated with smoking, such as age, clinical, dietary, anthropometric and demographic variables.[22,23] The present study did not assess in detail the health conditions of the individuals present in the sample. This bias could change the results, however it may have been minimized by the proposed exclusion criteria. Still, comorbid conditions that impair oxygen transport, such as anemia, heart disease and lung disease, are associated with cases of low-grade methemoglobinemia,[24] and, therefore, should be considered in future studies.

Still as a recommendation for future studies, arterial blood gases can be used for a more accurate measurement of oxygen saturation and also of the partial pressure of carbon dioxide. In addition, it is necessary to compare MetHb levels with complementary tests of inflammatory markers, such as C-reactive protein, xanthine oxidase and/or lactate dehydrogenase, and also the determination of total plasma antioxidant activity, or specific antioxidants, such as vitamin C or vitamin E, as cigarette consumption can decrease the concentration and activity of these markers and increase their systemic inflammation effects.[25] It is suggested to perform the laboratory dosage of these parameters and relating to the methemoglobin values, once that, to date, there are no statistical analysis studies that performed the comparison of reduced blood values of these antioxidant and inflammatory markers in smokers influencing in the methemoglobin values.

Conclusion

In the present study, the evaluation between groups of smoking and non-smoking volunteers did not detect alterations in methemoglobin saturation and hematological parameters, both in relation to clinical reference values and in the statistical analysis among groups. In the oxygen saturation, a higher mean was observed in smokers compared to the control group, being extremely important for future perspectives in research discussions focusing on the evaluation of detailed hemoglobin parameters presented in smokers. Further research is needed, as the topic lacks recent work with automated technologies. In addition, new studies with a larger sample group and the performance of additional determinations are also suggested, such as, for example, the relation methemoglobin with carboxyhemoglobin, or even the dynamic acute effect of cigarette smoke inhalation on hematological and methemoglobin parameters.

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Review article

Leprosy: A clinical review

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Abstract

Leprosy is a chronic infectious disease, caused by *Mycobacterium leprae*. It has great historical importance and is responsible for high rates of infected people all over the world, Brazil being the second country in number of cases, only behind India. The clinical manifestations of the disease depend on the host's immune response, and it has the potential to affect practically all organs and systems, although it mainly affects the skin and peripheral nerves, and may, above all, present periods of aggravation, which are called reactions. Thus, it is characterized as a disease of incapacitating character, which can bring irreparable physical deformities, presenting a great impact on the physical, social, and mental health of the patient. In this review we discuss the main aspects involving the condition of neglected disease, especially the epidemiology, classifications, clinical picture, complications, diagnosis, treatment, and care for prevention.

Keywords: *Mycobacterium leprae*; Leprosy; Epidemiology.

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Introduction

Leprosy is a chronic, neglected infection of clinical and historical importance, caused in most cases by *Mycobacterium leprae*. The disease is prevalent in Asia, Africa, and the Americas, especially in low- and middle-income countries. The number of annual reports exceeds 200,000 cases, so leprosy remains a major public health problem in endemic countries. *M. leprae* is also the cause of leprosy neuropathy, one of the most common infectious neuropathies worldwide.[1]

The various clinical manifestations of leprosy result from variations in the tissue response of genetically predisposed individuals to the presence of the etiologic agent and depending on the immune status of the patient, the bacteria die or multiply.[2] Disease transmission occurs via the respiratory route between infected and healthy individuals.[3] In addition, *M. leprae* DNA has been detected in soils near animal and human sources, indicating that such environments may represent temporary reservoirs of the bacterium, paving the way for studying other forms of transmission.[4]

Since leprosy is a neglected disease and Brazil is an important endemic area, it is necessary to conduct studies in order to understand the ongoing impact of the disease on affected individuals.[3] Additionally, developing, standardizing and deploying more accurate diagnostic tests in order to detect and treat it as early as possible and avoid the resulting disabilities is essential, especially among the most marginalized and less socially and economically advantaged individuals.[5] Therefore, important and current information about leprosy is presented here, with emphasis on the etiological, epidemiological, clinical and classification aspects, the complications, diagnosis, treatment and prevention of leprosy, which can help in the development of treatment plans and rehabilitation of patients.

Etiologic agent

The genus *Mycobacterium* consists of gram-positive *Actinobacteria* with high GC (guanine cytosine) content, which includes both non-pathogenic and pathogenic species.[6] *M. leprae* is an obligate intracellular pathogen, not grown on artificial media,[7] but grown *in vivo* in experimental animals,[8] which until 2008 was the only known etiological agent of leprosy. Another agent of the disease is *M. lepromatosis,* described in 2008 in Mexico in patients with fatal disseminated virchowian leprosy, and with DNA sequence distinct from *M. leprae*.[9] However, despite this considerable genetic divergence between these species, the clinical manifestations and treatment are similar, and it is only

possible to distinguish them using molecular tests.[10] *M. leprae* multiplies more slowly than *M. lepromatosis*, with a generation time of about 12 to 13 days,[11] which implies a long incubation period, which can range from two to 10 years after infection, depending on the form of the disease.[12] *M. lepromatosis* is predominant in cases of diffuse lepromatous leprosy (DLD), a severe form of the disease, endemic in Costa Rica and Mexico.[13] Molecular epidemiological studies about leprosy are useful to understand the focal transmission and the global spread of strains.[14] They can help to know transmission and to identify individuals who are at risk of contracting the disease.[15] In addition, molecular epidemiology allows a more adequate understanding of the evolution of the pathogenic strain associated with ancient human migrations and phylogeographic perceptions involving the spread of diseases worldwide.[10]

Globias, clusters of *M. leprae* adhered and bound by gelatinous substance, obtained from the lesions of untreated multibacillary patients, can be observed by light microscopy after Ziehl-Neelsen staining.[16] *M. leprae* shows tropism for Schwann cells, keratinocytes, and macrophages[17] and a predisposition to spread more efficiently in cooler regions of the body, such as the nerves near the skin surface, the skin itself, and the membranes of the upper respiratory tract.[11]

Humans are the main carriers of the infection, excluding the American continent, where nine-banded armadillos (*Dasypus novemcinctus*) also function as zoonotic reservoirs of *M. leprae*.[7] Indeed, in the United States of America, leprosy is recognized as a zoonosis, where contact with armadillos represents a significant risk for developing the disease.[18] In other countries, however, the role played by them is being investigated as a real possibility, given the reports of infected nine-banded armadillos in countries such as Colombia, Mexico, Argentina, French Guiana and even Brazil.[10] The presence of *M. leprae* in soils near animal and human activities has also been reported,[4] and the fact that bacilli remain viable inside amoebas for up to 35 days, even without replicating,[19] may be an indication of the contribution of these free-living protozoa in leprosy transmission. On the other hand, the detection of *M. lepromatosis* in red squirrels (Sciurius vulgaris) in Scotland, Ireland and England with lesions like those seen in leprosy,[10] allows us to question whether these animals were reservoirs of the pathogen and the possibility of contributing to the disease cycle.

In fact, the most common route of transmission results from contact with droplets released from the upper airways of individuals with multibacillary (MB) leprosy,[20] and more rarely through the skin or by vertical transmission.[7] However, the possibility of zoonotic infection is considered a challenge to the World Health Organization (WHO) paradigm when considering leprosy elimination, which includes strategies based only on interrupting transmission between individuals, excluding any approach involving zoonotic transmission.[7]

Close and prolonged contact, especially with multibacillary patients, especially men, individuals older than 60 years or in situations of social vulnerability is another factor that favours contagion and transmission of the disease.[20] Additionally, susceptibility to the bacillus depends on the immunosuppression, immunodeficiency and genetic predisposition of individuals.[16]

Epidemiology

Leprosy is prevalent in tropical and subtropical countries and is more common in developing countries due to its association with socioeconomic vulnerability indicators.[21] Although the prevalence of the disease has been decreasing since the institution of multidrug therapy in the 1980s, an important number of cases is described in many countries in the Americas, Africa, Southeast Asia, the Eastern Pacific and the Western Mediterranean.[3]

The disease is reported in about 143 countries, with 214,783 new cases, an average of 2.9 cases per 100.000 inhabitants. In Brazil, specifically, this rate is much higher (12.2/100,000 hab), second only to India and followed by Indonesia, Bangladesh and Nigeria,[21] with reported 80% cases.[3] In Latin America, Brazil accounts for over 90% of cases.[22] Worldwide, about 7.6% of new leprosy cases occur in children, and it can be interpreted that the disease has continuous active transmission in some communities.[10]

The most of cases in Brazil are described in males, people of colour, and lower education, coming from the Midwestern (37.27/100,000 inhab.) and Northern (34.26/100,000 inhab.) regions of the country, and at a lower frequency in the Southern (3.75 per 100,000 inhabitants) and Southeastern (5.31 per 100,000 inhabitants) regions.[16] Data from molecular epidemiology have shown the presence of several distinct strains of *M. leprae* circulating in the country, with a higher prevalence of two: SNP type 4 in the Southeast and SNP subtype 3I in the Northeast. Subtype 3I, more common in medieval Europe, is likely derived from multiple introductions by successive waves of colonization.[23]

Cases of leprosy (LLD) caused by *M. lepromatosis* are endemic to Costa Rica and Mexico, with smaller numbers recorded in Canada, Singapore, Brazil and Myanmar. Furthermore, this etiologic agent is also responsible for other clinical forms of the disease, which may coexist with *M. leprae* in these areas.[13]

Madrid classification

The Madrid classification (1953) considers the tendency of leprosy to evolve naturally towards one of two stable and diametrically opposed poles - Virchowian (VV) and tuberculoid (TT) - from two unstable groups - indeterminate (I) and dimorphic (D, or *boderline,* B), (Figure 1) that are characterized by specific signs and symptoms (Table 1). This is the most widely used classification in Brazil and is included in the notification forms of the Sistema de Informação de Agravos de Notificação (SINAN).[16] In this classification, the pure neuritic type is included in the indeterminate, lepromatous and tuberculoid groups, which is considered a problem.[2]

Figure 1. Evolution of the clinical forms of leprosy according to the Madrid classification

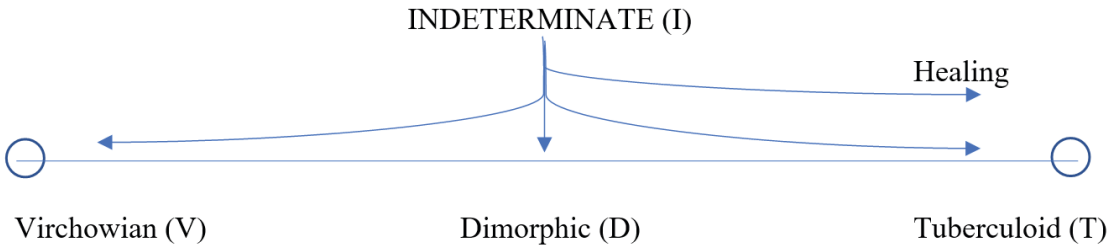


Table 1. Main characteristics of the clinical forms of leprosy according to the Madrid classification

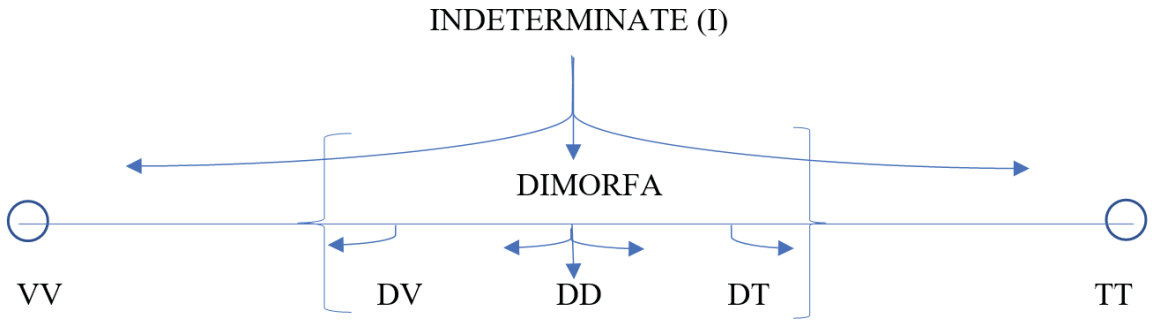
Form	Signs and symptoms	Mitsuda	Bacilloscopy	Peripheral neural involvement	Leprosy reaction
I	Hypochromic spot or hypoesthetic area, imprecise limits	Positive or negative	Negative	Absent	Absent
T	Erythematous or erythemato-hypochromic plaque, anesthetic, defined borders	Positive	Negative	Localized, close to the skin lesion	Absent
D	Hypochromic patches, erythematous plaques, anesthetic areas, foveolar lesions	Positive or negative	Positive or negative	It can be intense, early, multiple	Reaction outbreak type 1 (all) or type 2 (positive sputum smear)
V	Erythemato-violaceous plaques, livedo reticularis, nodules, infiltration, madarosis, xeroderma	Negative	Positive	Late, moderate, diffuse	Reactionary outbreak type 2

Source: Lyon S & Grossi MAF. Leprosy. Rio de Janeiro: Medbook, 2013 (adapted).[24]

Ridley & Jopling classification

The Ridley and Jopling[25] classification is based on the spectrum of clinical, histopathological and immunological features of the individuals. In this model, dimorphic leprosy is categorized into the Virchowian (LL) and tuberculoid (TT) forms at the ends of the spectrum and cases showing characteristics intermediate between the extremes (Figure 2). Thus, the dimorph group is subdivided into three subgroups: dimorphic-virchowian (DV), dimorphic-dimorphic (DD), and dimorphic-tuberculoid (DT).[26] This classification has been widely accepted, as is based on bacteriological, immunological, histopathological and clinical features of leprosy. However, it does not consider neuritic leprosy in the spectrum.[2]

Figure 2. Spectrum of clinical forms of leprosy by Ridley and Jopling classification



Source: The authors (2021).

In Ridley and Jopling's Classification,[25] the indeterminate form marks the beginning of the disease, and most cases tend to evolve spontaneously to cure. However, evolution to polarized or dimorphic forms is possible, which takes an average of five years, and may be earlier for the TT form. The lesions are typically characterized by a hypochromic macula with imprecise borders and the presence of sensitivity disturbances, areas of hypo- or anaesthesia. Usually few or single lesions are present, anhidrosis and/or hair loss is possible, and there is no peripheral nerve involvement. The smear is negative and the Mitsuda reaction positive or negative, which may indicate a tendency to evolve into the extreme forms. Histopathological findings include focal lymphohistiocytic, perivascular, perianexial, and perineural inflammatory infiltrates, but the diagnosis of leprosy is only made by the visualization of bacilli within the nerve filaments. Nevertheless, when the Mitsuda test is negative, this form is considered Paucibacillary (PB) for treatment purposes.[16]

The tuberculoid-tuberculoid (TT) form is more benign, due to the more effective cellular immune response against the bacillus. The lesions typically show as a macula or plaque, erythematous and/or hypochromic, with well-defined and slightly elevated borders, with reduced or lost sensation. The lesion is usually single or in small numbers (usually no more than three lesions). Symmetric nerve trunk involvement occurs, although asymmetric involvement is possible and may be the only clinical manifestation of the disease. In the area near the plaque lesion, neural thickening of the underlying nerve trunk ("Racket Sign") may occur,[27] and in the lesions themselves or in the nerve tracts with total loss of sensation, anhidrosis and/or alopecia.[16]

Alteration in sensitivity tends to follow an evolutionary order: (1) altered thermal sensitivity; (2) painful and (3) tactile. Histopathological findings include the observation of cohesive granulomas, consisting of epithelioid histiocytes and, occasionally, multinucleated giant Langhans-like cells, which is attributed to a more effective Th1 response. The granulomas tend to elongate, accompanying the vessels, nerves, and sweat glands, circumscribed by lymphocyte sheaths, with rare or absent bacilli. In addition, granulomas may permeate and destroy neural filaments. The smear is negative and the Mitsuda test positive. Additionally, the nodular form of childhood can occur, which is a variation of the T form. It affects children aged 1 to 4 years who have household contact with MB adults, forming erythemato-brown papular or nodular lesions on the face and limbs, usually single or small in number, without neural involvement, and regressing, leaving an area of atrophy. On histopathological examination, well-organized tuberculoid granulomas are observed.[16]

Another extreme form, Virchowian-virchowian (VV) is the most severe, due to the deficient cellular immune response against the bacillus, which results in multiplication of the bacteria within macrophages and Schwann cells. The patient with this null response is called anergic and presents diffuse tegumentary infiltration, abundant lesions in plaques, tubercles and nodules (leprosy), erythematous to violaceous in color, which can also affect the oral mucosa. In this form, the leonine facies may develop, characterized by auricular involvement with infiltration of the face (which gives the appearance of thickened skin and an enlarged nose) and superciliary and ciliary madarosis - with hair maintenance. Given the absence of cellular immune response, the bacilli become free to disseminate through the lymphatic and blood routes and reach other organs such as the lymph nodes, spleen, liver, adrenals, larynx,

bone marrow, testes, synovium, and eyeball. The more common features of the TT form – involving sensory disturbances and peripheral neural involvement – are present, but in a later and less marked form. There are two main variants: the histoid virchowian variant, which is defined by the formation of keloid-like lesions and may be sulfone resistant; and the Lucio variant, in which there is typically diffuse infiltration of the entire integument, making lesions more difficult to visualize. The Pike phenomenon can be frequent in these cases and is characterized by the formation of highly contagious necrotic ulcers. In addition, auto aggressive leprosy disease can appear in patients with the VV or, rarely, the DV form, and occurs given the development of autoimmunity - which explains the differential diagnosis of the condition with systemic lupus or rheumatoid arthritis. It is characterized by a febrile picture, anorexia and weight loss, arthralgia and neuralgia, which is succeeded by internal organ involvement and type II leprosy reactions, or even necrotizing vasculitis.[16,28]

The histopathological findings include many macrophages with ample or vacuolated cytoplasm, indicating that the bacilli were phagocytosed but could not be destroyed (since the Th1 response failed), and plasma cells, which are responsible for humoral immunity, with sparse lymphocytes. The epidermis is separated from the inflammatory infiltrate by the fibrous band of collagen known as the Unna band. The neural filaments are preserved, although they are surrounded by numerous bacilli and macrophages. Over time, the macrophages become foamy or vacuolated (Virchow cells), due to the accumulation of bacilli within them. In addition, mycobacteria can also be found in large numbers in blood vessels, in the piloerector muscles, and in the root sheaths of hair follicles. The IB can range from 5+ to 6+, the Mitsuda reaction is negative, and the smear is positive. Operationally classified as MB.[16,28]

The dimorphous-tuberculoid (DT), dimorphous-dimorphous (DD) and dimorphous-virchowian (DV) forms (Table 2) show immunological instability, with

Table 2. Characteristics of the dimorphic - tuberculoid (DT) form according to the Ridley and Jopling Classification (1962)

	Dimorphic - tuberculoid (DT)	Dimorphic - dimorphic (DD)	Dimorphic - virchowian (DV)
"Form"	Unstable; tends to the TT extreme.	Unstable; tends to one of the extremes, TT or VV.	Unstable, tends to the extreme VV.
Characterization of the lesions	Cutaneous similar to the TT form. They are usually satellites, smaller and more numerous around the main lesion.	Foveolar - eithymatopigmentary plaques, edematous; with a depressed nucleus, hypo- or normochromic or hypo- or anesthetic.	Cutaneous similar to the VV form, with nodules and infiltrations; more violaceous coloration, mainly on the face and in the ear pinnae. It may show as disseminated.
Histopathological findings	Milder, extensive granulomas limited to the dermis, with the formation of a narrow collagenous fibrous band just under the epidermis (Unna's band or Grenz's zone). Langhans-type multinucleated giant cells in varying numbers. Thickening of the nerves tends to be irregular, and usually the nerve filaments are more preserved.	Traces of loose, diffusely distributed granulomas, with the presence of clear cytoplasmic epithelioid cells. Absence of multinucleated giant cells. Diffusely distributed mimicked lymphocytes in number. Neural fillets are mostly easy to identify and many show proliferation of Schwann cells. Excessive interstitial edema occurs in the dermis.	Moderate activation of macrophages. Presence of numerous lymphocytes compared to the VV form, forming poorly defined granulomas. Presence of plasma cells can occur. Easily identifiable neural filaments that show proliferation of Schwann cells or perineural fibroblasts, ("onion skin" appearance).
Mitsuda's reaction	Positive	Generally negative	Negative
Bacilloscopy	Negative	It can be nositiva	Positive
IB	Can range from 0 to 2+	Can range from 3+ to 4+	Can range from 4+ to 5+
Operational Classification	Multibacillary	Multibacillary	Multibacillary

Source: Ridley DS, et al. (1966).[25] (Adapted); Brazil, Ministério da Saúde (2019.[16]

varied clinical presentations, which may approach the extreme poles, tuberculoid or virchowian. Skin lesions are varied, presenting as erythematous, erythema-to-hypochromic, brownish, ferruginous, infiltrative, edematous, shiny plaques or nodules, with altered or complete loss of sensation. Neurological involvement is frequent and may be more extensive and asymmetric, and neuritis may occur. In addition, this form is more prone to reactionary episodes (type I and II flare-ups), which can progress and lead to disability. Finally, the patient's skin is usually dry and anhydrotic, which predisposes it to cracks and ulcers. They are operationally classified as MB.[16,29]

Operational classification

The operational classification proposed by the WHO in the late 1990s divides the forms into paucibacillary (few bacilli - PB) and multibacillary (many bacilli - MB) and is used for treatment purposes. PB cases are those that result in up to five skin lesions, affect only one or none of the peripheral nerves, and are always smear negative. Patients with MB have more than five skin lesions and/or involve more than one peripheral nerve, and the smear may be negative or positive.[30] In Brazil, the Ministry of Health in 2002 guided the counting of the number of lesions as sufficient clinical criteria for operational classification, given the difficulty in diagnosing the neural thickening criterion, dependent on the assessment of examiners, and more difficult for those with less experience.[16]

Complications of leprosy

These are clinical manifestations of leprosy that do not fit into the typical categories of the disease such as primary nerve lesion and reaction episodes. Primary neural leprosy is a public health problem, especially in developing countries. It is a clinical form characterized by the absence of skin lesions and negative skin smears, so the diagnosis is based mainly on complementary tests, especially electroneuromyography, nerve biopsy, serology and molecular analyses. These more advanced tools are not always available in health services, even in those considered reference in the treatment of the disease.[31]

The most typical manifestations of primary nerve lesion involve the asymmetric involvement of nerves (multiple mononeuropathy), and the most affected nerves are the ulnar, median, radial, posterior tibial, and common peroneal nerves. The ulnar nerve lesion alone or together with the median nerve leads to the clinical sign of "claw hand", and if there is a radial nerve lesion, there is also the "fallen hand" deformity. Lesions in the tibial trunk, on the other hand, result in loss of sebaceous gland innervation and sensitivity in the plantar region, making the skin drier, more fragile, and susceptible to traumas that predispose to plantar perforating disease. The involvement of the common peroneal nerve makes it impossible to elevate the foot, making walking difficult, causing the "fallen foot" sign. In the face, lagophthalmos may occur, due to injury to the zygomatic branch in the facial nerve.[16] Nonspecific symptoms may include intense burning pain, paraesthesia, thermal and painful anaesthesia, hypohidrosis, motor losses, soft tissue involvement, arthritis in small and large joints, nerve thickening, and pain on palpation. The various differential diagnoses include rheumatoid arthritis, spondyloarthropathies, collagenosis, vasculitis, diabetes, hypothyroidism, tumours, AIDS, syphilis, traumatic causes.[32]

Ocular complications are important problem in Brazil. Multibacillary patients had a higher risk of developing lagophthalmos, that does not revert with the treatment and was associated with punctate keratitis, cataracts, uveitis and a higher risk of reduced visual acuity.[33] These ocular changes were frequently associated with neural disease and all patients must be evaluated by a specialist in ocular disease. These complications indicate the need of complete assistance to patients even after the resolution of the disease.[34]

Reaction episodes

Leprosy, characterized by a chronic course, can present reactionary outbreaks, i.e., acute or subacute manifestations due to immunological hyper-reactivity to *M. leprae* antigens.[35] Immunological events affect between 8 to 33% of patients,[36] with pictures that can be triggered by situations that alter the immune system: infections, vaccination, use of iodinated drugs, pregnancy and puerperium, physical, surgical or psychological stress situation or trauma.[35] During the natural evolution of the disease, concomitant to treatment or even after cure, reactions can show specific complications, which result in sequelae if not treated properly and quickly. Complications in the ocular apparatus cause scleritis, uveitis, iritis and iridocyclitis, with irreversible lesions and even blindness.[16]

Reaction episodes can be subdivided into type 1 reactions (T1R), with a predominance of high levels of tumour necrosis factor alpha (TNF-α), interferon-gamma

(IFN-α), interleukin-17 (IL-17) and chemokine 10, and erythema nodosum leprosum (T2R) (Antunes *et al.*, 2020). Type 1 reaction is more common in paucibacillary patients with the tuberculoid or dimorphic forms of the disease.[37] It usually starts before treatment with multidrug therapy (MDT) or within the first six months, but it can also be the first clinical manifestation of the disease. It is characterized by the appearance of new lesions or the re-aggravation and exacerbation of old lesions, with an erythematous and edematous appearance, and the formation of infiltrates or nodules that may develop into ulcers. Increased hypo- or anesthetic areas and neuritis, involving shock and/or pain in the neural tract, are common. Associated factors are edema of the hands and feet and the abrupt onset of "claw hand" and "foot drop," given the most common involvement of the ulnar, median, fibular, and tibial nerves.[17]

More recently, studies have suggested SARS-COV-2-induced hyperinflammation as the cause of mortality in leprosy patients. This is because the presence of the virus can trigger cytokine storm syndrome and patients express pro-inflammatory profile in the blood plasma with IL-2, IL-7, TNF-α, among others.[36]

Systemic involvement is not frequent, being restricted to the most severe cases, with fever, malaise, fatigue and anorexia. Hematological tests generally show no alterations, and when they do occur, the most common is leucocytosis. It is noteworthy that the reverse reaction must be differentiated from the relapse; the outbreak has a sudden and unexpected evolution, with old lesions reaggravated, and usually appears during treatment or up to five years after its end and responds well to therapy using corticoids. The relapse is rarer, has a slow and insidious onset, the old lesions are usually imperceptible, usually occurs five years after the end of multidrug therapy and does not respond well to corticotherapy.[17]Erythema nodosum leprosum is described in multibacillary patients, in the VV and some DV forms,[37] usually during or after treatment with MDT, but it can also be the first manifestation of the disease. It is an immune hyper-reactivity, which occurs in the blood and tissues, mainly in the skin, kidneys and joints,[36] caused by the deposition of immunocomplexes, which generate an intense inflammatory reaction. This clinical manifestation is more severe and can leave irreversible sequelae. Erythema nodosum leprosum is characterized by the sudden appearance of erythematous, subcutaneous, painful nodules, can evolve to vesicles, pustules, ulcers, and necrosis in severe cases, mainly on the face and upper and lower limbs. There is also a systemic involvement, and the occurrence of hepatosplenomegaly, glomerulonephritis, edema of the extremities – which can lead to epistaxis, orchitis, lymphadenitis, vasculitis, iridocyclitis, periostitis, nasal obstruction, scleritis, and episcleritis – and the gradual involvement of the nerve trunks is possible. The main associated symptoms are fever, asthenia, myalgia, nausea, joint pain, and weight loss.[36] Neuritis, when it occurs is less intense compared to that which occurs in type I reaction. Acute, diffuse inflammation of the soft tissues of the feet and hands cause serious edema and pain, are referred to as "reaction hands" or "reaction feet," and can leave sequelae. Erythema polymorphous may also be part of this leprosy reaction and is represented by erythematous, swollen, circular skin plaques that may resemble a target. Regarding laboratory tests, leucocytosis can occur, with deviation to the left, neutrophilia, platelets, increased immunoglobulins and proteinuria.[16] Regarding neutrophilia, in the pulmonary capillaries, SARS-COV-2 can trigger an extensive neutrophil infiltration, in severe patients. Similarly, patients with erythema nodosum leprosum, show intense perivascular infiltrate of neutrophils in the dermis, which makes some researchers argue that neutrophils, influenced by the presence of the virus, is related to the development of ENL (erythema nodosum leprosum) in leprosy patients.[36]

Some complications and sequelae of this reaction are in the case of acute orchitis, which can lead to testicular atrophy and the later appearance of gynecomastia. In addition, amyloidosis can be a complication of the VV form, with type 2 reactions. The differential diagnosis is sepsis.[16]

Diagnostics

The diagnosis of leprosy is essentially clinical and epidemiological, but complementary tests contribute to the clinical classification. Early diagnosis and specific treatment are essential to reduce sequelae, complications and to prevent transmission of the disease. In Brazil, the diagnosis is defined if the patient presents skin lesion with altered sensitivity or nerve involvement with neural thickening or positive bacilloscopy.[38]

The *polymerase* chain reaction (PCR) has high sensitivity and specificity; more than 90% and 100%, respectively.[11] The high cost restrict its use to research centres in Brazil.[24]

ELISA or rapid immunochromatographic tests show low sensitivity, and they are not recommended, especially for PB patients, who are mostly seronegative.[12]

Treatment

The treatment of leprosy is outpatient and should be carried out in basic health units, with multidrug therapy (MDT), recommended by WHO since 1982. MDT is a combination of rifampicin, dapsone and clofazimine or rifampicin and dapsone, used to treat patients with MB (Table 3) and PB (Table 4), respectively.[39] With treatment, transmission ceases and cure is assured,[11] however, in fertile women there may be interaction between rifampicin and contraceptives.[40]

Paucibacillary cases, in which there is a single lesion on the skin, are treated with the ROM scheme in a single dose - rifampicin at a dose of 600 mg, ofloxacin at a dose of 400 mg, and minocycline at a dose of 100 mg. In special situations where adherence to standard treatment is difficult, such as in mental disorders and alcoholism, monthly supervised doses can be used, six doses for PB and 24 for MB.[16]

In children and adults with a body mass of less than 30kg, the doses are adjusted according to the patient's mass (Table 5).

Table 3. Treatment regimens for multibacillary (MB): 12 frames in up to 18 months

Adult	Rifampicin (RFM): monthly dose of 600 mg (2 capsules of 300 mg) with supervised administration.
	Dapsone (DDS): supervised monthly dose of 100 mg and a self-administered daily dose of 100 mg.
	Clofazimine (CFZ): monthly dose of 300 mg (3 capsules of 100 mg) with supervised administration and a daily self-administered dose of 50 mg.
Child	Rifampicin (RFM): monthly dose of 450 mg (1 capsule 150 mg and 1 capsule 300 mg) with supervised administration.
	Dapsone (DDS): supervised monthly dose of 50 mg and a self-administered daily dose of 50 mg.
	Clofazimine (CFZ): monthly dose of 150 mg (3 capsules of 50 mg) with supervised administration and a 50 mg dose self-administered every other day.

Source: Brazil, Ministry of Health (2019).[16]

Table 4. Treatment regimens for paucibacillary (PB): 6 tablets in up to 9 months

Adult	Rifampicin (RFM): monthly dose of 600 mg (2 capsules of 300 mg) with supervised administration.
	Dapsone (DDS): supervised monthly dose of 100 mg and self-administered daily dose of 100 mg.
Child	Rifampicin (RFM): monthly dose of 450 mg (1 capsule 150 mg and 1 capsule 300 mg) with supervised administration.
	Dapsone (DDS): supervised monthly dose of 50 mg and self-administered daily dose of 50 mg.

Source: Brazil, Ministry of Health (2019).[16]

Table 5. Doses for MDT adjustment according to the patient's body mass

Dapsone (DDS)	**Rifampicin** (RFM)	**Clofazimine** (CFZ)
1.5 mg/kg/day	10 mg/kg/day	1 mg/kg/day (daily dose) 5 mg/kg/day (monthly dose)

Source: Brazil, Ministry of Health (2019).[16]

The introduction of World Health Organization (WHO) multidrug therapy (MDT) has played a pivotal role in achieving the epidemiological target of elimination of leprosy as a public health problem.[41] However, a subset of MB patients who not responding satisfactorily (clinically and microbiologically) to the current fixed duration (FD) of WHO-MDT-MB regimen (MBR) is observed. In Brazil, the drug treatment currently offered by the Sistema Único de Saúde (SUS) combines three drugs: rifampicin, dapsone and clofazimine. In cases of resistance to rifampicin, the Ministry of Health recommends the exchange for minocycline or ofloxacin. For cases that, however, there is resistance to treatment, there has been the therapeutic option of using clarithromycin since December of 2020.[42]

Narang *et al,* observed in India in patients who are "nonresponsivenes" to conventional treatments after anti-leprosy therapy comprised minocycline 100 mg/day, clofazimine 50 mg/day, and ofloxacin 400 mg/day for 6 months (intensive phase), and ofloxacin 400 mg/day and clofazimine 50 mg/day for the next 18 months (maintenance phase). They conclude that treatment is safe and effective in the management of MB leprosy patients who are nonresponsive to 12 months of WHO-MDT-MBR.[41]

Prevention and control

The prevention and treatment of leprosy and reaction states seek to prevent the transmission of the disease, the onset of disabilities and permanent physical impairments, as well as to avoid further emotional and socioeconomic damage to the patient.[16] Thus, to reduce the disease burden, it is necessary to strengthen government control, coordination and partnership, combat leprosy and its complications, combat discrimination and promote social inclusion. Emotional support by family, community and health workers is essential, but no less important than social and financial support services.[21]

The main way to prevent sequel of the disease is early diagnosis and immediate initiation of treatment.[38,43] For this, it is necessary to systematically search for patients in endemic areas, investigate household or close contacts of the patient and offer free, immediate and specific treatment.[44]

Smith[45] observed that the use of chemoprophylaxis with dapsone and acedapsone is effective in reducing the incidence of leprosy, particularly among home contacts, who have a higher risk of developing disease. Chemoprophylaxis for contacts is promising, and the drug of choice is now the rifampicin,[46] which can prevent up to 57% of cases in the first two years.[47]

Vaccination with BCG (Bacille de Calmette et Guérin), although specific for tuberculosis, show efficacy against leprosy and is indicated for contactantes intradomiciliary.[48] Nevertheless, there are two vaccines (*Mycobacterium indicus pranii (*MiP) and LepVax) under study that may standardize post-exposure prophylaxis.[46,49]

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Review article

Relationship between vitamin D and cancer: A narrative review

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Abstract

Introduction: Vitamin D is involved in several human metabolism pathways, both in classic pathway, which involves the calcium and phosphorus metabolism, and in non-classic pathways, which are related to several types of diseases, including diseases related to muscles; kidneys; cardiovascular system; immune system; some types of cancer; diabetes; and pregnancy. Objectives: As cancer is one of the main health problems in the world and knowing that it is one of the non-classic effects related to vitamin D, the current narrative review aimed to verify the relevance of vitamin D has on the types of cancer. Methods: The bibliographic research was performed in databases Pubmed, Scopus and PEDro on June 16, 2020, using the keywords "vitamin D", "cancer" and "non-classic". Only articles since the year 2000 were included. Thirty-one articles were analyzed relating vitamin D to colon/rectum cancer, breast cancer, prostate cancer, lung cancer, ovarian cancer, melanoma and other types of skin cancer, and gastric cancer. Results: All studies have shown a relationship between vitamin D and the incidence of cancer in the human body, however, there are peculiarities regarding the concentration levels in each organ. Even with conflicting results, in general, vitamin D has shown to be promise in the prevention of several types of cancer.

Keywords: Vitamin D; Cancer; narrative review; cancer prevention; health problems.

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Introduction

An important function of vitamin D is the maintenance of the balance of calcium and phosphorus metabolism in the human body.[1] There are two main forms of vitamin D: vitamin D3 (or cholecalciferol) that forms on the skin through sun exposure or ultraviolet light, and vitamin D2 (or ergocalciferol) obtained by irradiating plants or vegetable food.[1] They may be obtained through sun exposure, food intake or supplementation.[2] The first observation that uses the natural vitamin D originates from the 19th century in which rickets patients were successfully treated and cured with fish oil rich in vitamin D. In 1890 there was the first demonstration that exposure to sunlight could be effective in the treatment of rickets. But it was only in 1922 the first time that these anti-rickety properties derived from fish oil were linked with a fat-soluble factor first identified as vitamin D, and from then more studies involving this newly discovered hormone emerged.[3] To be biologically activated and have effects on metabolism and physiological functions, such as inhibiting the growth of cancer cells and protecting against certain immune mediated disorders, vitamin D must be converted into its active form.[4]

The sun emanates electromagnetic radiations that can be classified according to their frequencies and wavelengths, and some of them are infrared rays, visible light and ultraviolet radiation (UVR). UVR is found in the electromagnetic spectrum between wavelengths of 100 nm and 400 nm and is subdivided into ultraviolet A (UVA), ultraviolet B (UVB) and ultraviolet C (UVC)[5] Exposure to UVR has physiological and physical benefits, as the participation in synthesis of active vitamin D. The UVB is responsible for the formation

of pre-cholecalciferol (pre-vitamin D3) which will later be isomerized in cholecalciferol (vitamin D3).[6]

Both vitamin D2 and vitamin D3 circulate in the bloodstream to bind with a specific protein, the vitamin D binding protein (DBP). To become active, vitamin D is transformed twice; first a hydroxylation occurs forming 25-hydroxyvitamin D (25(OH)D) in the liver, this hepatic hydroxylation is not tightly regulated, so if the production of vitamin D is high, either by ingestion or exposure to the sun, the liver will produce more 25(OH)D, this pre-hormone will also bind to BPD with a half-life of 3 weeks. This pre-hormone can be hydroxylated in the kidney within the cells of the proximal tubule to form 1,25-dihydroxyvitamin D (1,25(OH)2D3), also known as calcitriol, which is the active metabolite of vitamin D; this hydroxylation is strongly regulated and is stimulated mainly by parathyroid hormone (PTH), low serum calcium and phosphate concentrations, and inhibited by fibroblast growth factor 23 and calcitriol itself. When released into the bloodstream, calcitriol is also able to bind to DBP, however with a lower affinity that 25(OH)D, being able to bind to various tissues that have the vitamin D receptor (VDR), which can activate or inhibit the transcription of several genes.[7]

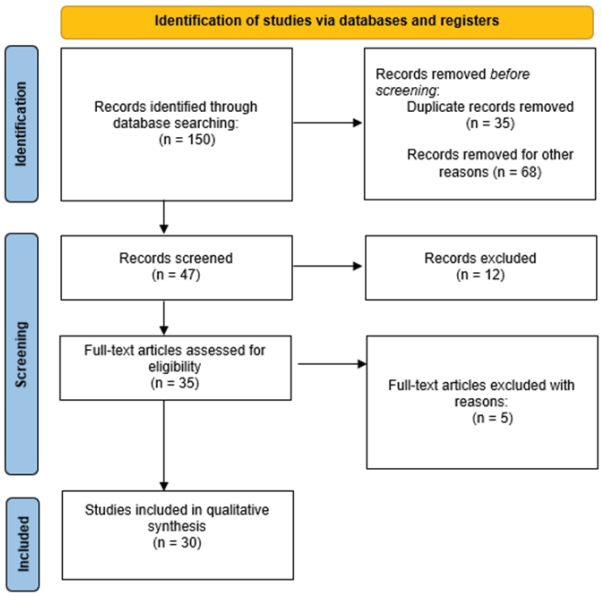
Several tissues have VDR and the mechanism capable of activating and deactivating vitamin D, even though they are not involved with bone and/or calcium and phosphate metabolism. 25(OH)D enters these tissues and it is transformed locally into calcitriol, which will bind to the cell's VDR generating several genomic effects (not "calcemic"), thus not participating in the metabolism of calcium and phosphate. This pathway does not appear to be regulated by PTH or by the fibroblast growth factor 23, but by the extracellular concentration of 25(OH)D shrouded these tissues. Thus, one can understand the non-classic effects of vitamin D, which are considered "intracranial", when compared to the classic effects which are endocrine. The non-classic effects, most common in the body, are related to the muscles; cardiovascular system; kidneys; immune system; some types of cancer; diabetes; and pregnancy.[8]

Cancer is a health problem that affects developed and underdeveloped countries, including the main causes of morbidity and mortality worldwide, with 18.1 million new cases and 9.6 million deaths in 2018, causing damage to patients, families, community and the health system. Knowing that one of the non-classic effects of vitamin D is related to some types of cancer and that this disease is the second leading cause of global death,[9] the current study aimed to conduct a narrative review to verify effects of vitamin D on types of cancer.

Methodology

For this narrative review, the bibliographic research was carried out on Pubmed, Scopus and PEDro on June 16, 2020. The keywords selected for the searches were "vitamin D", "cancer" and "non-classic". Only articles published since the year 2000 were used. The PRISMA[10] was used to guide the development and organization through a diagram flow (Figure 1).

Figure 1. Flow diagram according the findings in the databases



Inclusion criteria

In this narrative review were included nineteen Pubmed articles, twelve Scopus articles and no PEDro articles. The inclusion criteria randomized studies from the year 2000.

Exclusion criteria

Non-randomized and non-meta-analysis studies, articles published prior to 2000, animal clinical trials, studies with vitamin D concomitant with another substance, studies with vitamin D and classical effects and studies with vitamin D and other non-classical effects were excluded.

Results and discussion

A total of thirty articles were analyzed, of which seven related vitamin D with colon/rectum cancer,

three with breast cancer, three articles with prostate cancer, three with lung cancer, two with cancer in the ovaries, one related to melanoma and other types of skin cancer, three related to gastric cancer and eight with anticarcinogenic properties of vitamin D.

The relationship between vitamin D and cancer risk

Colorectal Cancer

Four meta-analyzes published in 2011 that demonstrated the inverse association between vitamin D levels and the risk of getting colorectal cancer, in other words a high level of 25(OH)D would be associated with low risk of developing this type of cancer.[11-13] Another study corroborated this information and also found that increased serum levels of vitamin D plays a key role in decreasing the risk of colorectal adenoma.[14] High levels of circulating vitamin D also decreased the methylation of SFRP2 (a not-invasive biomarker for diagnosis of colorectal cancer) in the tumor area and these results suggests that vitamin D has an epigenetic effect on DNA methylation, contributing to a positive response as a neoadjuvant treatment.[15]

A randomized clinical trial also demonstrated that high doses of vitamin D were able to slow the growth rate of colorectal cancer in patients with chemotherapy started.[16] Messaritakis et al.[17] conducted a study that was able to demonstrate that therapies which activate the vitamin D receptor, including the modulation of this pathway, may be a new approach for the treatment of colorectal cancer, according to the thought that vitamin D would be beneficial in the treatment of this type of disease.

Breast Cancer

The investigation involving breast cancer and vitamin D are much less consistent than those related to colorectal cancer, however a work by Shao et al.[18] concluded that a high concentration of circulating 25(OH)D is associated with a low risk of developing this type of cancer. Bauer et al.[19] also conducted a dose-response meta-analysis and found a non-linear inverse association, only in a more restricted group composed by women in the post-menopausal period.

Zhou et al.[20] did not identify in their meta-analysis any protective effect of vitamin D supplementation in reducing the risk of breast cancer, and this may be related to several factors such as an inadequate dosage when compared to the control group, short-term follow-up, patient selection and even unknown factors. This study suggests the complexity surrounding the relationship between vitamin D and breast cancer risk, and this may differ with menopausal status and possibly between races as well.

Prostate Cancer

Studies concluded that men with elevated serum levels of 25(OH)D had a higher risk of developing prostate cancer, compared to men with low levels of the vitamin.[21] Nair-Shalliker et al.[22] suggested that the elevated level of 1,25(OH)2D on plasma after prostate cancer diagnosis and treatment could decrease all specific causes of mortality from this disease, especially in men with aggressive prostate cancer, and also suggested that circulating vitamin D levels post-diagnosis can be used as a prognosis for survival from aggressive prostate cancer.

Torkko et al.[23] did not find a clear or consistent association with the vitamin D pathway and its interactions with 25(OH)D present in the blood, in relation to the risk of prostate cancer. However, they identified that patients with low amounts of 25(OH)D in the serum have a greater chance of developing prostate cancer with a risk of being fatal.

Lung Cancer

Two researches with slightly different criteria have been published reporting the relationship between vitamin D levels and the risk of lung cancer. Zhang et al.[24] included only prospective studies and observed a statistically lower risk of lung cancer, comparing high and low categories of circulating vitamin D. Chen et al.[25] also concluded that there was a 5% reduction in the risk of lung cancer with administration of 10 nmol/L of 25(OH)D.

Kong et al.[26] evaluated the relationship between the single nucleotide polymorphism related to the vitamin D pathway and the prognosis of non-small cell lung cancer (NSCLC). It was noted that some of the genetic polymorphisms related to the vitamin D pathway can influence the prognosis of lung cancer and may be linked to tumor progression, which may indicate vitamin D as a potential therapeutic possibility.

Ovarian Cancer

Despite not having significant statistical relevance, some prospective studies of circulating vitamin D and ovarian cancer suggested a reduced risk of developing

the disease associated with a high level of 25(OH)D.[27] Circulating vitamin D levels are significantly lower in patients with ovarian cancer, so vitamin D deficiency is closely related to the pathogenesis of ovarian cancer. Vitamin D treatment reduces the migration and proliferation of ovarian cancer cells and its use should be considered as a potential new therapy for ovarian cancer.[28]

Melanoma and other types of skin cancer

There is a high complexity involving vitamin D and skin cancer, considering that exposure to ultraviolet radiation is both a source of vitamin D and a risk factor for this type of cancer. No association was found between skin cancer and increased vitamin D through food or supplementation, not confirming that a high in vitamin D is associated with cutaneous melanoma. Although vitamin D inhibits the development and spread of the tumor in the early stages of the disease, it is still unclear whether it has an effect in later stages and whether it can increase the survival of patients with melanoma.[29]

Stomach Cancer

Vitamin D is related to the risk of gastric cancer[30] and metabolites or analogues of vitamin D can suppress *Helicobacter pylori*[31] infection and H. pylori-related gastric cancer. The protective role of vitamin D has been identified against this type of human cancer through several studies[30] and has shown that vitamin D suppresses proliferation and stimulates cell cycle arrest in gastric cancer cells.[32]

Anticarcinogenic properties of vitamin D

It is suggested that vitamin D can regulate the process of tumor formation, from the beginning to interactions with the cell.[33] This mechanism includes the cell regulation, such as proliferation, differentiation, apoptosis, autophagy and even the interaction of the cell with the environment in which it is inserted (angiogenesis, inflammation, among others).

The process of tumoral formation is capable of changing irreversible genetic mutations in cells, leading to several transformations mainly related to cell function, and vitamin D in turn participates in key points in the prevention of this whole process, playing an important role of antioxidant, anti-inflammatory defense and without DNA repair process. One of the greatest contributors to the onset of tumor formation is inflammation and works suggest that vitamin D can exert at least four different anti-inflammatory mechanisms:[34] Vitamin D in its active form, that is calcitriol, can inhibit the prostaglandin (PG) pathway, which in turn is involved in the process of repairing tissue damage and infection. Calcitriol inhibits the pathway by inhibiting the expression of cyclooxygenase-2 (COX-2) and PG receptors, and is also involved in the degradation of prostaglandin itself.[35] Vitamin D can also suppress the MAPK p38-mediated proinflammatory signaling pathway by inducing MAPK phosphatase-5 expression, which prevents MAPK p38 phosphorylation and activation, inhibiting the production of proinflammatory cytokines such as interleukin-6 (IL-6).[36] Calcitriol can also inhibit the nuclear factor kappa B (NFkB) signaling pathway that performs functions as a transcription factor. It suppresses protein kinase B (AKT) phosphorylation and its target in macrophages, by the positive regulation of member 4 of the superfamily thioesterase (THEM4), an AKT modulator. This leads to inhibition of NFkB and COX-2 expression.[37] Vitamin D is also able to regulate the interaction between immune cells and tumor cells to suppress the production of pro-inflammatory cytokines.[38]

In addition to inflammation, reactive oxygen species (ROS) are important points in various stages of tumor formation, which can promote DNA mutation, cell proliferation and death, all of which provoke pro-inflammatory responses. So, maintaining a good antioxidant defense system is a critical point in preventing the development of tumors. Studies suggest that vitamin D is able to protect the cell against oxidation induced by DNA damage by promoting antioxidant defenses.[39,40]

The vitamin D-mediated protection from DNA damage that is induced by ROS can be attributed to the process of inducing the expression of several enzymes involved in the body's antioxidant barrier, such as SOD1, SOD2, GSH, NRF2, among others. In addition to the increase in antioxidant capacity, vitamin D is also able to directly regulate the DNA repair process,[40] increasing the expression of genes involved in this process.

The current narrative review has some limitations and therefore its results should be interpreted with caution. Only cohort studies that were published from the year 2000 on were included, the searches were performed only in three databases and in the English language.

The strength of this work is related to the presentation of a relationship between vitamin D and the presence of cancer in various organs.

Conclusion

Vitamin D insufficiency is frequently shown to be related to the incidence of cancer in the human body. It is possible to observe the existence of peculiarities regarding the difference in levels of concentration of vitamin D in each organ. Even with conflicting results, in general, vitamin D has shown promise in the prevention of several types of cancer.

Conflict of interest

The authors have no potential conflicts of interest in publication of this study.

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Canvas: A case report

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Abstract

CANVAS is an acronym for cerebellar ataxia, neuropathy and vestibular areflexia syndrome that was recently described in 2011. This paper reports a case of a 69 years old female that presented a history of dizziness for 9 years, associated with altered vestibulo-ocular reflex (VVOR) and bilateral hypofunctiom at video head impulse test (vHIT), wicth confirmed CANVA syndrome. Imbalance is a consistent symptom of CANVAS and, in most cases, it is the first to manifest. Its presentation demonstrates the importance of the specialist's need for knowledge of this clinical condition to enable an assertive diagnosis.

Keywords: CANVAS; Vestibular areflexia; Vídeo head impulse test; Vestibulo-ocular reflex; Dizziness; Vestibulo hypofunction.

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Introduction

CANVAS, an acronym for cerebellar ataxia, neuropathy and vestibular areflexia syndrome, was first described in 2011 by Szmulewicz and collegues, who also proposed the diagnostic criteria for CANVAS in 2016. The most important findings are: clinical evidence of bilateral vestibular hypofunction, clinical evidence of cerebellar impairment (cerebellar atrophy on magnet resonance image (MRI) and/or signs of cerebellar impairment on examination), and abnormal nerve conduction testing that is consistent with a sensory deficit but excludes nerve entrapment neuropathies or other known pathology (neurophysiologic evidence of a neuronopathy (ganglionopathy).[1,2]

Most cases of CANVAS are diagnosed on an outpatient basis, as the characteristic clinical sign, altered vestibulo-ocular reflex (VVOR – visually enhanced vestibulo-ocular reflex), is simple to be identified in the office and recorded by video-oculography.[1] It can only be obtained if both smooth-pursuit eye movements and the vestibulo-ocular reflex are deficient. In addition to this manifestation, patients with this syndrome have sensory deficits on nerve conduction studies as evidenced by abscent or reduced sensory nerve action potentials (SNAPS), in addition to central changes such as cerebellar atrophy, which involves the cerebellar vermis and lateral hemispheric atrophy (consistent pattern of anterior and dorsal vermis atrophy, the latter involving vermal lobules VI, VIIa, and VIIb. Laterally, a pattern of hemispheric atrophy predominantly affecting crus I (corresponding to vermal lobule VII) was seen),[3] identified through MRI of the skull.[4]

The genetic disorder in CANVAS was discovered to be an abnormal biallelic expansion in the replication factor C subunit 1 (RFC1), an autosomal recessive inherited disease.[5,6]This pathological expansion was found in 100% of the familial form, and 92% of seemimgly sporadic ones when the triad was complete.[7] This mutation has been identified as a major cause of late-onset ataxia, which affects the cerebellum, sensory ganglia including the vestibular system, and explaining the pathology of CANVAS.[5,8]

Case report

DLSS, 69 years old, female, started with dizziness in 2012, previously diagnosed with PPPD (persistent perceptual postural dizziness), due to the dizzying condition associated with great emotional stress, provoked by the death of her mother.

Only in 2020 the diagnosis of CANVAS was confirmed after dizziness recurrence, when the patient sought a specialized service in otorhinolaryngology.

She reported unspecific dizziness with imbalance and light headness whenever she was standing. She also had sensorineural hearing loss in both ears (presbycusis), without tinnitus. On physical examination, bilateral corrective saccade to the head impulse test (HIT) was observed and difficulty in performing the Romberg and Fukuda test.

After initial evaluation, complementary exams were requested. The video head impulse test (vHIT) revealed bilateral hypofunctiom (Figure 1), the visually enhanced VVOR was also abnormal (Figures 2 and 3) and posturography revealed changes in the static balance and in the integration of the balance systems (somatosensory, vestibular and visual) (Figure 4). These tests were all performed in a specialized clinic.

After the results of the complementary exams, which confirmed bilateral labyrinth hypofunction, the patient was referred to a neurologist for evaluation of neuropathy and cerebellar ataxia. After physical examination, the speciallist confirmed the cerebelar ataxia, that demonstrated pendular patellar reflex, finger-nose dysmetria, normal elevation of limbs with eyes closed and gait with extended steps (drunken gait), complemented by the aid of an electroneuromyography examination of the upper and lower limbs (ENM), with axonal degeneration. Then, magnetic resonance imaging (MRI) was requested, which showed cerebellar atrophy.

At the moment, the patient has been in vestibular rehabilitation for 3 months, with improvement of vestibular symptoms.

Figure 1. Video Head Impulse Test showing hypofunction of all semicircular canals in both ears

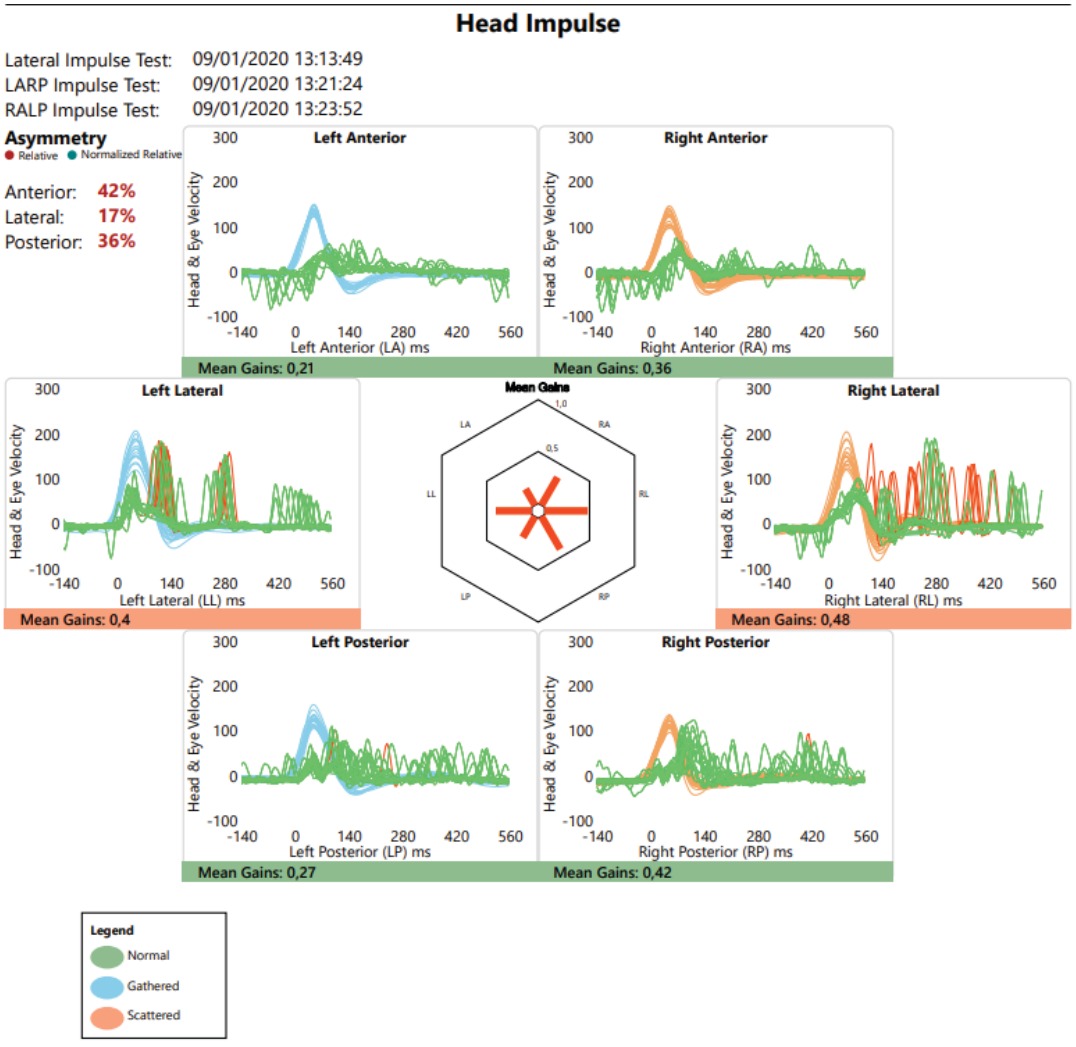


Figure 2. refixation balances during horizontal rotation of Altered visually enhanced vestibulo-ocular reflex (VVOR): the head at approximately 1 Hz

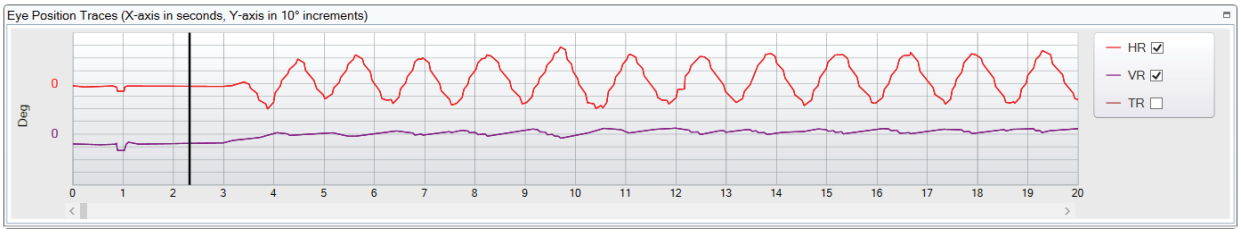


Figure 3. Altered visually enhanced vestibulo-ocular reflex (VVOR): refixation balances during horizontal rotation of the head at approximately 1 Hz

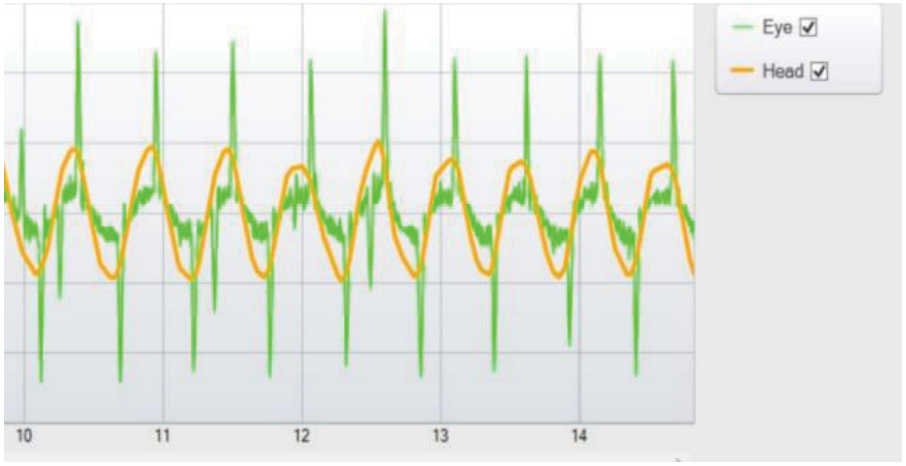
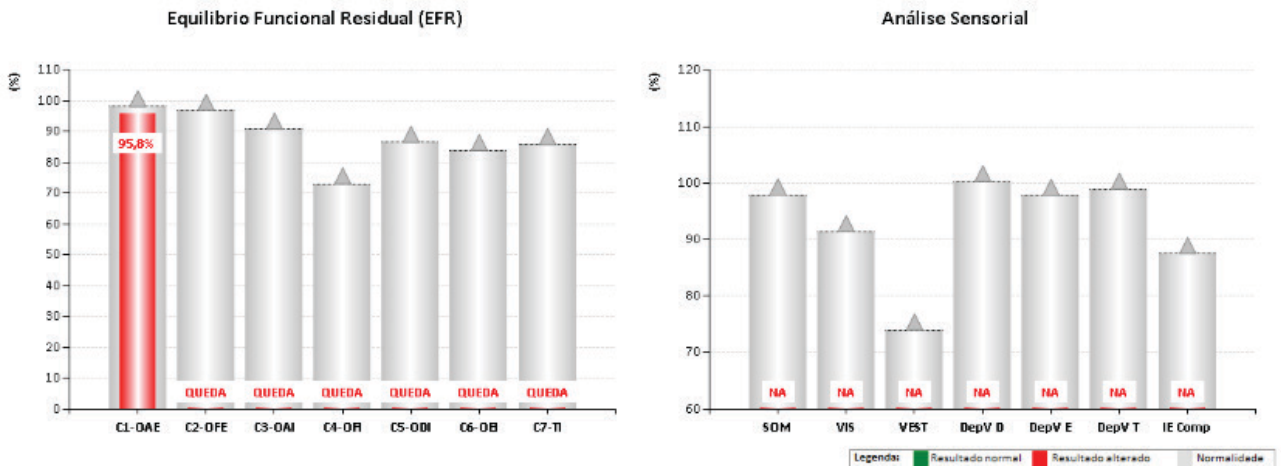


Figure 4. Posturography showing alteration in the integration of all pathways of balance (somatosensory, visual and vestibular)



Discussion

Vestibular areflexia and sensory loss in CANVAS syndrome have been attributed to a sensory neuronopathy that affects the dorsal root and V, VII, and VIII cranial nerve ganglia. Histopathological studies of the temporal bone in post-mortem patients showed severe impairment of Scarpa's ganglion with a reduction in the number of ganglion cells bilaterally as a consequence, bilateral vestibular nerves were also atrophied, as they originated in the ganglion in question[9,10]

The auditory component of the vestibular nerve was intact, in post-mortem analysis, with the vestibular terminal organs (ridges and macules) unchanged.[10] Unexpectedly, the facial nerves may be atrophied, especially in the geniculate ganglion, in addition to the altered trigeminal ganglion with an important decrease in the cellular component. The neuropathology of these patients shows loss of Purkinje cells (predominantly in the vermis and lateral cerebellum)[9]

Imbalance is a consistent symptom of CANVAS and, in most cases, it is the first to manifest, although sometimes it develops as the syndrome progresses.[1] The characteristic oculomotor sign of this syndrome is the clinical evidence of an abnormal visually enhanced VVOR. The VVOR assessment is done by demonstrating clinically the presence of compensatory rather than smooth saccadic eye movements, moving the head from side to side while the patient looks at a fixed target, and represents combined cerebelar and bilateral vestibular impairment.[1,11] The opticokinetic reflex occurs when, in the presence of an entire scene that oscillates from side to side, different from the small target in the smooth search, compensatory eye movements are produced to fix the image on the retina.[12]

A diagnostic challenge is to make sure that a patient who has one of the CANVAS triad (cerebelar ataxia, bilateral vestibulopathy and a somatosensory deficit) has also a second, or, indeed, a third component.

There are some differential diagnoses for CANVAS, the most important include: spinocerebellar ataxia (SCA3), multiple system atrophy with predominant cerebellar ataxia (MSAc), idiopathic cerebellar ataxia and bilateral vestibulopathy (iCABV), and Wernecke's encephalopathy.[13]

The case report fulfills all the criteria of the syndrome, with proof through complementary exams. Both VHIT and VVOR show bilateral labyrinthic areflexia (Figures 1, 2 and 3), posturography (Figure 4) with changes in all balance systems (visual, somatosensory and vestibular), ENM with axial degeneration, physical examination compatible with cerebellar ataxia and sensory deficit, in addition to skull MRI which shows cerebellar atrophy. As the patient in question does not have the RFC1 gene test positive or at least the SCA3 gene test negative, therefore we can affirm that it is highly suspisious for CANVAS.

Conclusion

In recent years much has been studied about CANVAS, including its clinical manifestations, diagnostic and histopathological criteria.[2,10,11] Despite this, it is still a little known syndrome in medical practice. Its presentation, associated with otorhinolaryngological signs and symptoms, demonstrates the importance of the specialist's need for knowledge of this clinical condition to enable an assertive diagnosis

The follow up by the neurologist and otorhinolaryngologist is to control vestibular symptoms by vestibular rehabilitation. The prognosis of the CANVAS is still reserved, studies are needed to better elucidate the clinical management.

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Normas para publicação

Submissão de artigos – Brazilian Journal of Health and Biomedical Sciences

O ***Brazilian Journal of Health and Biomedical Sciences*** **(BJHBS)**, inicialmente denominado Revista HUPE, publica artigos inéditos sobre temas diversos em todas as áreas relacionadas às ciências da saúde e biomédicas, e que não estejam simultaneamente em avaliação em nenhum outro periódico. Este jornal rejeita prontamente as práticas de plágio e autoplágio. É composto de seções dedicadas a estudos originais, revisões, estudos de caso e cartas ao editor. Os textos são aceitos em um dos três idiomas: português, inglês e espanhol. O processo de submissão de manuscritos deve considerar as seguintes orientações:

Avaliação por pares: os manuscritos são revisados por pelo menos dois especialistas no assunto. Aqueles que forem aceitos serão editados de acordo com o padrão editorial do **BJHBS**, com o objetivo de melhorar a clareza e eliminar possíveis redundâncias, sem, contudo, alterar o sentido original. A versão final editada será submetida aos autores para aprovação.

Termo de transferência de direitos autorais/conflitos de interesses: após o aceite final do artigo para publicação, os autores deverão enviar o termo de transferência dos direitos assinados pelo autor principal representando cada um dos autores. Neste termo deverão ser declarados quaisquer conflitos de interesses.

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- uma declaração de que o manuscrito não foi submetido para publicação em outro periódico;
- recomendação de dois consultores qualificados que sejam especialistas na área científica do artigo submetido (informando *e-mail* e afiliação). O Conselho Editorial poderá escolher algum destes consultores ou não;
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  Se os autores receberam ajuda de escritores técnicos ou revisores de idiomas quando prepararam o manuscrito, isto deve ser explicitado na carta de apresentação, junto com a declaração de que os autores são totalmente responsáveis pelo conteúdo científico do manuscrito.

  Informação da autoria: o mérito da autoria científica deve ser limitado aos participantes que contribuíram intelectualmente no trabalho, além de uma colaboração efetiva para a realização da pesquisa. Portanto, para ser considerado um autor, cada colaborador deve, no mínimo, preencher as seguintes condições: (a) ter contribuído significativamente na concepção e desenho do estudo, ou na análise e interpretação dos dados; (b) ter contribuído substancialmente na elaboração do artigo, ou revisado criticamente o seu conteúdo intelectual e (c) ter aprovado sua versão final a ser publicada. A supervisão/coordenação geral do grupo de pesquisa por si só não justifica a autoria. A contribuição somente na aquisição de verbas provenientes de fontes financiadoras ou na coleta de dados também não é suficiente para justificar autoria. A fim de garantir a transparência dessas informações, os autores são solicitados a incluir uma declaração a respeito da autoria, descrevendo o papel dos autores no estudo e na preparação do manuscrito. A falta desta declaração sobre autoria na carta de apresentação implicará na desconsideração do artigo para avaliação.

  A carta deverá ser assinada pelo autor principal, que representará os demais autores neste documento.

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- nome de cada autor com a respectiva afiliação, nesta ordem: prenome, nomes intermediários abreviados, último sobrenome. Departamento (ou serviço). Faculdade. Universidade (ou instituição). Cidade, UF, país.
- dados de contato do autor correspondente: prenome, nomes intermediários abreviados, último sobrenome, endereço de correspondência, *e-mail*.

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1. Artigos originais:

Artigos que resultam de pesquisas originais. São limitados a 5.000 palavras (excluindo resumo e referências) e um máximo de cinco figuras ou tabelas. A lista de referências é limitada a 40 itens. Devem ser apresentados no seguinte formato:

- **resumo:** deve ser apresentado em português, inglês e espanhol, limitado a 250 palavras. Deve seguir o modelo de resumo estruturado, contendo, obrigatoriamente: introdução, objetivos, materiais e métodos, resultados e conclusões. Sabe-se que o resumo alcança maior visibilidade e distribuição do que o artigo em si, por isto deve conter as informações essenciais do artigo, mas não deve ser uma simples composição de frases copiadas do texto integral.

  Deve ser sucinto e objetivo, destacando o que há de mais importante no texto, com o objetivo de atrair o leitor para a leitura integral. Ao final, a conclusão deve constar a relação dos resultados obtidos com os objetivos estabelecidos para o estudo. Adicionalmente, faz-se necessário apontar as contribuições desses resultados para o conhecimento acerca do tema pesquisado.
- **descritores**: devem ser apresentados três a seis termos referentes ao tema apresentado, separados por ponto e vírgula, conforme os Descritores em Ciências da Saúde (DeCS), para os idiomas português e espanhol, ou o Medical Subject Headings (MeSh) para o idioma inglês.

Texto do artigo:

- **introdução**: deve ser curta e conter o propósito (contexto e justificativa) do estudo, incluindo um breve resumo dos estudos relevantes sobre o assunto em questão, citando seus avanços mais recentes, citando apenas as referências pertinentes.
- **materiais e métodos**: esta seção deve constar, resumidamente, as informações que permitam que o estudo seja replicado por outros pesquisadores. Os procedimentos adotados devem ser descritos claramente, bem como as variáveis analisadas, com as respectivas definições sempre que necessário, além da descrição da hipótese a ser testada. Devem ser descritas a população e a amostra, instrumentos de medida, com a apresentação, se possível, de medidas de validade, e conter também informações sobre a coleta e processamento de dados. Deve ser incluída a devida referência para os métodos e técnicas empregados, inclusive os métodos estatísticos. Métodos novos ou substancialmente modificados devem ser descritos, justificando as razões para seu uso e mencionando suas limitações. Os critérios éticos da pesquisa devem ser respeitados. Os autores devem explicitar que a pesquisa foi conduzida dentro dos padrões éticos e aprovada por comitê de ética.
- **resultados**: essa seção deve ser um relato conciso da nova informação descoberta, com o mínimo de julgamento pessoal. Devem ser apresentados numa sequência lógica, iniciando pela descrição dos dados mais importantes. Não se deve repetir nos textos os dados de tabelas e ilustrações, mas apresentá-los resumidamente.

Normas para publicação

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- **agradecimentos**: devem ser registrados de forma concisa e limitados àquelas pessoas e/ou instituições que contribuíram para a pesquisa de alguma forma, mas não puderam ser incluídos como coautores.
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3. Artigos de revisão:

Devem versar sobre temas relevantes para a prática médica. Estes artigos comporão a seção que versará sobre o tema comum daquela edição, na área de saúde. São limitados a 5.000 palavras (excluindo resumo e referências) e um máximo de cinco figuras e/ou tabelas. A lista de referências é limitada a 40 itens. Os trabalhos serão submetidos à avaliação do conselho de editores via convite ao editor que organizará esta seção, conforme regras que seguem:

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- **descritores**: devem ser apresentados três a seis termos referentes ao tema apresentado conforme lista dos Descritores em Ciências da Saúde (DeCS), para os idiomas português e espanhol ou do Medical Subject Headings (MeSh) para o idioma inglês. Devem ser separados por ponto e vírgula.

Os artigos de revisão poderão ser de dois tipos:

a. **Revisão sistemática e meta-análise** – Por meio da síntese de resultados de estudos originais, quantitativos ou qualitativos, o artigo deverá responder à pergunta específica e de relevância para os artigos sobre o tema da edição, no contexto da área de saúde (ver foco do **BJHBS**). Descreverá de modo detalhado o processo de busca dos estudos originais, os critérios utilizados para seleção daqueles que foram incluídos na revisão e os procedimentos empregados na síntese dos resultados obtidos pelos estudos revisados (que poderão ou não ser procedimentos de meta-análise).

b. **Revisão narrativa/crítica** – A revisão narrativa ou revisão crítica possui caráter descritivo-discursivo, e visa à apresentação abrangente e à discussão de temas de interesse científico na área de saúde. Deve apresentar formulação clara de um objeto científico de interesse, argumentação lógica, crítica teórico-metodológica dos trabalhos consultados e síntese conclusiva. Deve ser elaborada por pesquisadores com experiência no campo em questão ou por especialistas de reconhecido saber.

- Agradecimentos: devem ser registrados de forma concisa e limitados àquelas pessoas e/ou instituições que contribuíram para a pesquisa de alguma forma, mas não se encaixam nos critérios estabelecidos para os coautores.
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o número de autores for três ou mais, deve-se citar o primeiro autor acrescido da expressão "e colaboradores": "Silveira e colaboradores[13] propuseram uma nova metodologia...".

- Referências: todas as referências citadas no texto deverão compor a lista de referências. As referências devem ser restritas a material publicado, artigos ou resumos. Os autores são responsáveis por preencher as referências de modo preciso e completo. Para referências com mais de um autor, deve-se listar até três autores por extenso, acima disso, deve-se listar os três primeiros autores seguidos de ", et al.". O total de referências não deve exceder 40.
- Tabelas e/ou figuras: deverão somar no máximo cinco.
- Tabela: deve ser elaborada com programas apropriados, tais como o Excel, podem ter a largura proporcional à largura de uma página diagramada, considerando fonte Arial de tamanho 9, espaçamento simples. Devem ser submetidas em arquivo de texto: DOC (Microsoft Word), RTF (Rich Text Format) ou ODT (Open Document Text). Numeradas em ordem crescente e acompanhadas de título e/ou legenda explicativa, com uma referência objetiva no texto. Em nenhuma situação o conteúdo de uma tabela deverá replicar o de uma figura ou vice-versa. Devem ser numeradas em ordem crescente com números arábicos, em conforme o aparecimento no texto.
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Editorial: trata-se de um comentário e análise relativa a um artigo na edição em questão. Pode incluir uma figura ou tabela e limita-se a 750 palavras, com até cinco referências. Será elaborado pelo editor e/ou algum colaborador convidado por ele.

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Todas as comunicações subsequentes deverão ser feitas através da opção "responder" deste *e-mail* original.

O comitê editorial fará a avaliação do manuscrito de acordo com a linha editorial da revista e retornará a respeito do aceite para avaliação por pares no menor prazo possível. Caso ele seja considerado adequado para publicação, de acordo com a política editorial do **BJHBS**, entrará no fluxo editorial e passará pelas etapas de revisão textual e diagramação.

Após o aceite do artigo deverá ser enviado o termo de transferência dos direitos autorais e declaração de conflitos de interesses.

A prova de prelo será encaminhada para avaliação final em formato .pdf antes da efetiva publicação do texto e deverá retornar no prazo estabelecido pela equipe editorial.

Os textos e artigos que não atenderem às especificações descritas nestas normas serão devolvidos sem prévia avaliação pelo conselho de editores do **BJHBS**. Esses textos deverão ser resubmetidos ao processo de avaliação.

Paper submission – Brazilian Journal of Health and Biomedical Sciences

Brazilian Journal of Health and Biomedical Sciences (BJHBS), formely titled **Revista HUPE**, publishes new articles about several themes all related to health and biomedical sciences, provided they're not in simultaneous analysis for publication in any other journal. It rejects promptly any plagiarism and selfplagiarism practices. It features dedicated sections to original research, literature reviews, case studies, and letters to the editor. Papers must be submitted in one of three languages: Portuguese, Spanish, and English. The submission process comprises the following steps:

Peer review: papers are reviewed by at lest two specialists. Accepted papers will be edited according to the publishing standards of **BJHBS**, to improve readability and minimize redundancy, without loss of original meaning. The final edited version will be sent to authors for approval.

Copyright/conflicts of interest agreement: after the final approval, authors must send the copyright transfer agreement signed by the first author representing each additional author. In this agreement must be stated any conflicts of interest.

Introduction letter: a letter that must come with the submitted paper and contains at least the following information:

- a statement that the paper has not been submitted for publication in another journal;
- recommendation of two specialists for consulting in the scientific field of the submitted paper. The Editorial Board may or may not choose any of these consultants;
- conflicts of interest statement: state if the authors have any conflicts of interest. Conflicts of interest are those with potential influence over the published content, compromising the objectivity, integrity, or perceived value of the paper;
- author information: to provide full name and institutional affiliations of every author and a mailing address of the main author (postal and e-mail). Authors will be required to objectively state that the submitted paper consists of original content, informing it has not been previously published nor is it being analysed with this intent elsewhere.

  If the authors had assistance from technical writers or language reviewers, it must be explicitly stated in the introduction letter, along with the assurance that the authors are fully responsible for the scientific content of the paper.

  Authorship information: scientific authorship must be limited to those who contributed with intellectual work, with actual collaboration in the research. Therefore, to be considered an author, each contributor must meet the following conditions: (a) significant contribution to the creation and design of the study or to the analysis and interpretation of its results; (b) substantial contribution to the production of the paper, or critical review of its intellectual content, and (c) approval of the final version for publication. Leading or supervising a research lab/group does not in itself qualify as authorship. Sole contributions to fund raising or to data gathering also do not qualify as authorship. To ensure transparency in this aspect authors are expected to include a statement of authorship detailing the role of each author in the study and in the production of the paper. In

the absence of this authorship statement within the introduction letter, the paper will be disqualified for analysis.

The letter must be signed by the main author, who will represent all other authors in this document.

Title page: this page must contain title and author information as follows:

- title (in Portuguese, English, and Spanish) 100 characters maximum, counting spaces;
- short title (in Portuguese, English, and Spanish) 50 characters maximum, counting spaces;
- 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.
- contact information for an author: first name, abbreviated middle names, last name, mailing address, e-mail.

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:

- **abstract**: must be written in Portuguese, English, and Spanish, with a maximum of 250 words. Must follow the structured abstract model, with mandatory introduction, methodology and resources, results and discussion. It is well known that the abstract gets more visibility and distribution than the full text of the paper. Therefore, it must contain 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 DeCS (Descritores em Ciências da Saúde) for Portuguese and Spanish, and also 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 analysed 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 an 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.
- **acknowledgments**: must be concise and limited to people and institutions that contributed to the research in some degree, but could not be included as authors.
- **in-text citations**: **BJHBS** follows the Vancouver style, according to the general rules of The NLM Style Guide for Authors, Editors, and Publishers, second edition (www.ncbi.nlm.nih.gov/books/NBK7256/). For in-text citations, use Arabic numerals superscript,[1] without spaces, right after a word or punctuation: "Parkinson's Disease[1] description began in the 1950s,[2] when..." In some cases, the names of the authors may figure in the text: "Phillips[12] analysed several conditions of..."; and up to two authors can be named: "Handel and Matias[15] conducted a study about..." However, when the number of authors is three or more, the first author must be named along with the expression "et al": "Silveira et al[13] have proposed a new methodology..."
- **references**: all referenced cited in-text must be in the reference list. References are limited to published material, papers, and abstracts. Authors are responsible for providing precise and complete references. In references with more than one author, authors up to three must be named. From there on, an "et al" must follow the first three authors. There must be no more than 40 references.
- **tables and/or images**: up to a maximum of five.
- **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.
- **images**: can be photos, illustrations, graphics, drawings, etc. Images must be submitted as separate files (.tiff or .jpeg). 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

2. **Clinical cases**:

a. **Case report**: usually describes one to three patients or a family case. The text must be up to 2,000 words long, with up to three tables or images and up to 25 references. The abstract must be no more than 100 words long.

b. **Clinical case solution**: must contain a step by step description of the decision process of clinical cases. Patient information must be presented to one or more clinical experts in stages (text in bold) to simulate the way information is made available in clinical practice. The expert must answer (text in regular font) as new information is added, sharing their reasoning/arguments with the reader. The text must be up to 2.500 words long, and must have up to 15 references.

3. **Literature review**:

Must be about subjects relevant to medical practice. These will form a section about the common theme of each issue. These are limited to 5,000 words (excluding abstract and references) and a maximum of five images and/or tables. Maximum of 40 listed references. Literature reviews will be submitted for the editorial board analysis under invitation by the guest editor of this section, and must conform to the following standards:

Title page: this page must contain title and author information as follows:

- title (in Portuguese, English, and Spanish) 100 characters maximum, counting spaces;

- short title (in Portuguese, English, and Spanish) 50 characters maximum, counting spaces;
- 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.
- contact information for an author: first name, abbreviated middle names, last name, mailing address, e-mail.

Full text:

- **abstract**: must be written in Portuguese, English, and Spanish, with a maximum of 250 words for each language. Must follow the structured abstract model, with mandatory introduction, methodology and resources, results and discussion. It is well known that the abstract gets more visibility and distribution than the full text of the paper. Therefore, it must contain 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 according to DeCS (Descritores em Ciências da Saúde) for Portuguese and Spanish, and also MeSh (Medical Subjects Headings) for English. Keywords must be separated by semicolons.

Literature reviews may fall into two types:

a. **Systematic review and meta-analysis** – Through a synthesis of original studies' results, the paper must answer specific relevant health sciences questions about the theme of its issue (see **BJHBS'** focus). It must detail the search process to find the original studies, selection criteria, and synthesis procedures for the results of the reviewed studies (which may or may not be meta-analysis procedures).

b. **Narrative/critic review** – Narrative or critic review has a descriptivediscoursive character, and aims to offer a broad presentation and to discuss themes of scientific interest within the health field. It must have a clear formulation of the scientific subject of interest, a theoretical-methological critic of the reviewed works, and a conclusive synthesis. It must be elaborated by experienced researchers in the field in question or by renowned experts of notorious knowledge.

- **Acknowledgments:** must be concise and limited to people and institutions that contributed to the research in some degree, but could not be included as authors.
- **In-text citations: BJHBS** follows the Vancouver style, according to the general rules of The NLM Style Guide for Authors, Editors, and Publishers, second edition (www.ncbi.nlm.nih.gov/books/NBK7256/). For in-text citations, use Arabic numerals superscript,[1] without spaces, right after a word or punctuation: "Parkinson's Disease[1] description began in the 1950s,[2] when..." In some cases, the names of the authors may figure in the text: "Phillips[12] analysed several conditions of..."; and up to two authors can be named: "Handel and Matias[15] conducted a study about..." However, when the number of authors is three or more, the first author must be named along with the expression "et al": "Silveira et al[13] have proposed a new methodology..."
- **References:** all referenced cited in-text must be in the reference list. References are limited to published material, papers, and abstracts. Authors are responsible for providing precise and complete references. In references with more than one author, authors up to three must be named. From there on, an "et al" must follow the first three authors. There must be no more than 40 references.
- **Tables and/or images:** up to a maximum of five.
- **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.
- **Images:** can be photos, illustrations, graphics, drawings, etc. Images must be submitted as separate files (.tiff or .jpeg). 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

4. **Other submissions:**

Editorial: it's a commentary on or analysis of papers in a given issue. It may include an image or table and be no more than 750 words long, containing up to five references. It will be written by the editor in chief or by an invited contributor at their request.

Letters to the editor: space for reader's to talk about recently published papers. Each letter must have up to 200 words (excluding references), five references and one image or table. It must be submitted no later than six months after the publication of the relevant paper. Letters non-related to papers published by **BJHBS** are limited to 500 words (excluding references), five references, and one image or table. Authors of letters will be required to provide their details, as well as contact information and possible conflicts of interest. The decision about the publication of a letter is made by the editor in chief.

5. **On-line submission:**

Papers and other types of material must be sent to submission.bjhbs@hupe.uerj.br, along with the introduction letter. The subject of the e-mail must be: "Type of paper [original paper, case report, literature review]" or "Letter to the editor" -- title" + last name of its main author in UPPER CASE.

All subsequent communication must happen through responses to the original e-mail.

The editorial committee will analyse the material according to the editorial policies of **BJHBS** and will answer regarding acceptance for peer review as soon as possible. If it's considered fit for publication, it will be processed and proceed to editing, proofreading and layout.

After a paper's acceptance, the term of copyright transfer and the statement of conflicts of interest must be sent as soon as possible.

The final layout will be forwarded to the authors for final approval in .pdf format. This approval must be given according to a deadline defined by the editorial team.

Papers and other texts that do not conform to the specifications of these guidelines will be returned without any analysis by the editorial board of **BJHBS**. Such material must be re-submitted for new analysis once specifications are followed.

Normas para publicação

Sumisión de artículos - Brazilian Journal of Health and Biomedical Sciences

El ***Brazilian Journal of Health and Biomedical Sciences*** **(BJHBS)**, anteriomente titulado **Revista HUPE**, publica artículos inéditos sobre diversos temas relacionados con el área de ciencias de la salud y biomédicas que no estén siendo considerados simultáneamente en ninguna otra revista. Rechaza prontamente cualquieres práctica de plagio y/o de auto plagio. Está compuesta por secciones dedicadas a estudios originales, revisiones, estudios de caso y cartas al editor. Los textos son aceptados en uno de los tres idiomas: portugués, inglés y español. El proceso de sumisión de manuscritos debe considerar las siguientes pautas:

Evaluación por pares: los manuscritos son revisados por lo menos por dos especialistas en la materia. Aquellos que fuesen aceptados serán editados de acuerdo con la norma editorial del **BJHBS**, con el objetivo de dar más claridad y eliminar posibles redundancias, sin que eso quiera decir alterar el significado original. La versión final editada será sometida a los autores para aprobación.

Declaración de transferencia de los derechos de autor/conflictos de interés: después de la aceptación final del artículo para la publicación, los autores deberán enviar la declaración de transferencia de los derechos, firmada por el autor principal en representación de cada uno de los autores. En esta declaración deberán ser mencionados cualesquiera conflictos de interés.

Carta de presentación: una carta que deberá acompañar obligatoriamente el artículo sometido, conteniendo, por lo menos, las siguientes informaciones:

- una declaración de que el manuscrito no fue sometido para publicación en otra revista;
- recomendación de dos consultores calificados que sean especialistas en el área científica del artículo sometido (informando correo electrónico y entidad). El Consejo Editorial podrá escoger cualquiera de estos consultores o no;
- declaración de conflictos de interés: informar si los autores poseen o no algún conflicto de interés. Los conflictos de interés tienen el poder de influenciar el contenido de la publicación evitando la objetividad, integridad o percepción del valor de la publicación;
- declaración de autoria: proporcionar el nombre completo y las entidades de todos los autores y la dirección de contacto del autor para correspondencia (dirección, dirección de correo electrónico). También se solicita a los autores declarar objetivamente que el manuscrito sometido es material original, además de informar que no fue publicado anteriormente y que no está siendo valorado para publicación en ningún otro lugar.

  Si los autores recibieron ayuda de escritores técnicos o revisores de idiomas cuando prepararon el manuscrito, esto debe ser explicitado en la carta de presentación, junto con la declaración de que los autores son totalmente responsables por el contenido científico del manuscrito.

Información de la autoria: el mérito de la autoria científica debe limitarse a los participantes que contribuyeron intelectualmente en el trabajo, mas allá de una colaboración efectiva para la realización de la investigación. Por lo tanto para ser considerado un autor, cada colaboradordebe como mínimo cumplir con las siguientes condiciones: (a) haber contribuido de manera significativa en la concepción y diseño del estudio, o en el análisis e interpretación de los datos; (b) haber contribuido sustancialmente en la elaboración del articulo o revisado críticamente el contenido intelectual y (c) haber aprobado la versión final a ser publicada. La supervisión/coordinación general del grupo de investigación por si sola no justifica la autoria. Solo la contribución en la adquisición de sumas de dinero provenientes de fuentes financiadoras o en la recogida de datos tampoco es suficiente para justificar la autoria. Con el fin de garantizar la transparencia de esas informaciones, se solicita a los autores incluir una declaración al respecto de la autoria, describiendo el papel de los autores en el estudio y en la preparación del manuscrito. La falta de esta declaración sobre autoria en la carta de presentación implicará la desconsideración del artículo para valoración.

La carta deberá ser firmada por el autor principal, que representará a los demás autores en este documento.

Página del título: esta página deberá contener las informaciones del título, autores, de la siguiente manera:

- título (en portugués, inglés y español) de hasta 100 caracteres con espacio;
- título abreviado (en portugués, inglés y español) de hasta 50 caracteres con espacio;
- nombre de cada autor con su respectiva afiliación, en este orden: nombre, segundos nombres abreviados, último apellido. Departamento (o servicio). Facultad. Universidad (o institución). Ciudad, Unidad de la Federación (UF), país.
- datos de contacto del autor correspondiente: nombre, segundos nombres abreviados, último apellido, dirección de correspondencia, correo electrónico.

Tipos de artículos

1. Artículos originales:

Los artículos resultantes de investigaciones originales. Se limitan a 5.000 palabras (excluyendo resumen y referencias) y a un máximo de cinco figuras o tablas. La lista de referencias se limita a 40 ítems. Deben presentarse en el siguiente formato:

- **resumen:** debe presentarse en portugués, inglés y español, limitado a 250 palabras. Debe seguir el modelo de resumen estructurado, incluyendo, obligatoriamente: introducción, materiales y métodos, resultados y discusión. Se considera que el resumen alcanza una mayor visibilidad y distribución que el articulo en si, por esto debe contener las informaciones esenciales del artículo, pero no debe ser una simple composición de frases copiadas del texto integral.

  Debe ser sucinto y objetivo, destacando lo que es más importante en el texto, con el objetivo de atraer al lector a leerlo íntegramente. Al final, la conclusión debe comentar la relación de los resultados obtenidos con los objetivos establecidos para el estudio. Adicionalmente, es necesario hacer alusión a las contribuciones de esos resultados en el conocimiento acerca del tema investigado.
- **palabras clave:** deben presentarse de tres a seis términos concernientes al tema presentado, separados por punto y coma, conforme con las palabras clave en Ciencias de la Salud (DeCS), para los idiomas portugués y español, o el Medical subject Headings (MeSh) para el idioma inglés.

Texto del artículo

- **introducción:** debe ser corta y contener el objetivo (contexto y justificación) del estudio, incluyendo un breve resumen de los estudios relevantes sobre el tema en cuestión, citando los avances más recientes, citando apenas las referencias pertinentes.
- **materiales y métodos:** esta sección debe constar de, brevemente, las informaciones que permitan que el estudio sea replicado por otros investigadores. Los procedimientos adoptados deben ser descritos claramente, así como las variables analizadas, con las respectivas definiciones siempre que sea necesario, más allá de la descripción de la hipótesis a prueba. Deben describirse la población y la muestra, los instrumentos de medida, con la representación, si es posible, de las medidas de validez, y contener también informaciones sobre la recogida y procesamiento de datos. Debe incluirse la debida referencia para los métodos y técnicas empleados, inclusive los métodos estadísticos. Los métodos nuevos o sustancialmente modificados deben ser descritos, justificando las razones para su uso y mencionando sus limitaciones. Los criterios éticos de la investigación deben ser respetados. Los autores deben aclarar que

la investigación se llevó a cabo dentro de los patrones éticos y aprobada por el comité de ética.

- **resultados:** esa sección debe tener una descripción concisa de la nueva información descubierta, con el mínimo juicio personal. Deben presentarse en una secuencia lógica, a partir de la descripción de los datos más importantes. No debe repetirse en los textos los datos de tablas e ilustraciones, sino presentarlos resumidamente.
- **conclusiones:** debe mencionar el significado de la nueva información y la relevancia de los nuevos hallazgos en comparación con la literatura científica y las teorías existentes. En este apartado deben mencionarse las limitaciones del trabajo y también las implicaciones para las investigaciones futuras. Por último, las conclusiones deben ser parte del final, relacionandola con los objetivos citados en la introducción.
- **agradecimientos:** deben registrarse de forma concisa y limitarse a aquellas personas y/o instituciones que contribuyeron en la investigación de alguna forma, pero que no pudieron ser incluidos como coautores.
- **citaciones en el texto:** el **BJHBS** adopta el estilo Vancouver, siguiendo las normas generales de los Requisitos Uniformes para los Manuscritos presentados a Periódicos Biomédicos (www.ncbi.nlm.nih.gov/books/NBK7256/). Para citaciones en el texto, use números arábigos sobrescritos,[1] sin espacio, después la palabra y después la puntuación si hubiera: "La descripción de la enfermedad de Parkinson[1] ataca a los años de 1950,[2] cuando...". En algunos casos, los nombres de los autores pueden aparecer en el texto: "Phillips[12] valoró diversos cuadros de...", y deben citarse en el texto hasta dos autores: "Handel y Matias[15] hicieron un estudio sobre...". Sin embargo, cuando el número de autores es de seis o más, se debe citar al primer autor añadiendo la expresión "y colaboradores": "Silveira y colaboradores[13] propusieron una nueva metodología...".
- **referencias:** todas las referencias citadas en el texto deberán constar en la lista de referencias. Las referencias deben restringirse a material publicado, artículos o resumes. Los autores son responsables de llenar las referencias de modo preciso y completo. Para referencias con más de un autor, debe enumerarse los nombres por completo de hasta seis autores, por encima de esto, se debe enumerar los seis primeros autores seguidos de ", *et al.*". El total de referencias no debe exceder 40.
- **tablas y/o figuras:** deberán sumar como máximo cinco.
- **tabla:** debe elaborarse con programas apropiados, tales como Excel, pueden tener el ancho proporcional al ancho de una página diagramada, teniendo en cuenta el tamaño de fuente Arial 9, espacipo simple. Se deben entregar en archivo de texto: DOC (Microsoft Word), RTF (Rich Text Format) ou ODT (Open Document Text). Numeradas en orden ascendente y acompañadas por título y/o subtítulo explicativo, con una referencia objetiva en el texto. En ningún caso el contenido de una tabla deberá copiar el de una figura o vice-versa. Deben numerarse en orden creciente con números arábigos, conforme aparezcan en el texto.
- **figuras:** pueden ser fotos, ilustraciones, gráficos, diseños etc. Deben enviarse en archivos separados (formato *tiff ou JPEG). Se deben numerar en orden creciente con números arábigos, conforme su aparición en el texto.

2. **Casos clínicos:**

a. **Reporte de casos:** suele describir de uno a tres pacientes o una familia. El texto se limita a 2.000 palabras, un total de tres tablas y/o figuras y hasta 25 referencias. El resumen debe ser de 100 palabras.

b. **Solución de caso clínico:** ese texto debe contener los pasos del proceso decisorio de casos clínicos. Las informaciones del paciente deben presentarse a uno o más experto (s) clínico (s) por etapas (texto en negrita) para imitar el modo en el que la información se presenta en la practica clínica. El clínico debe hacer lo mismo (texto en fuente normal) conforme con las recientes informaciones adicionadas, compartiendo las justificaciones/argumentos con el lector. El texto debe tener hasta 2.500 palabras y 15 referencias.

3. **Artículos de revisión:**

Deben tratar temas de interés para la practica médica. Estos artículos comprenden la sección que abordará el tema común de aquella edición, en el área de salud. Se limitan a 5.000 palabras (excluyendo resumen y referencias) y a un máximo de cinco figuras y/o tablas. La lista de referencias se limita a 40 ítems. Los trabajos serán sometidos a la valoración del consejo de editores por medio de invitación al editor que organizará esta sección, conforme con las reglas que siguen:

Página del título: esta página deberá contener las informaciones del título, autores, de la siguiente manera:

- título (en portugués, inglés y español) de hasta 100 caracteres con espacio;
- título abreviado (en portugués, inglés y español) de hasta 50 caracteres con espacio;
- nombre de cada autor con su respectiva afiliación, en este orden: nombre, segundos nombres abreviados, último apellido. Departamento (o servicio). Facultad. Universidad (o institución). Ciudad, Unidad de la Federación (UF), país.
- datos de contacto del autor correspondiente: nombre, segundos nombres abreviados, último apellido, dirección de correspondencia, correo electrónico.

Texto del artículo:

- **resumen:** debe presentarse en portugués, inglés y español, limitado a 250 palabras. Debe seguir el modelo de resumen estructurado, con introducción, materiales y métodos, resultados y discusión. Se considera que el resumen alcanza una mayor visibilidad y distribución que el artículo en si, por esto debe contener las informaciones esenciales del artículo, pero no debe ser una simple composición de frases copiadas del texto integral. Debe ser sucinto y objetivo, destacando lo que es más importante en el texto, con el objetivo de atraer al lector a leerlo íntegramente. Al final, la conclusión debe incluir la relación de los resultados obtenidos con los objetivos establecidos para el estudio. Adicionalmente, es necesario hacer alusión a las contribuciones de esos resultados en el conocimiento acerca del tema investigado
- **palabras clave:** deben presentarse de tres a seis términos concernientes al tema presentado conforme con la lista de las palabras clave en Ciencias de la Salud (DeCS), para los idiomas portugués y español, o del Medical subject Headings (MeSh) para el idioma inglés. Se deben separar por punto y coma.

Los artículos de revisión podrán ser de dos tipos:

a. **Revisión sistemática y meta-análisis** - A través de la síntesis de resultados de los estudios originales, cuantitativos o cualitativos, el artículo deberá responder a la pregunta específica y de relevancia para los artículos sobre el tema de la edición, en el contexto del área de salud (véase el enfoque del **BJHBS**). Describe en detalle el proceso de búsqueda de los estudios originales, los criterios utilizados para la selección de los que se incluyeron en la revisión y los procedimientos empleados en la síntesis de los resultados obtenidos de los estudios revisados (que pueden ser o no procedimientos de meta-análisis).

b. **Revisión narrativa/crítica** - La revisión narrativa o revisión critica posee carácter descriptivo-discursivo, y se destina a la presentación exhaustiva y a la discusión de temas de interés científico en el área de salud. Debe presentar la formulación clara de un objeto científico de interés, argumentación lógica, crítica teórica y metodológica de los trabajos consultados y síntesis final. Debe ser elaborado por investigadores con experiencia en el campo en cuestión o por especialistas de conocimientos reconocidos.

- **Agradecimientos:** deben registrarse de forma concisa y limitarse a aquellas personas y/o instituciones que contribuyeron en la investigación de alguna forma, pero que no corresponden con los criterios establecidos para los coautores.

Normas para publicação

- **Citaciones en el texto:** el **BJHBS** adopta el estilo Vancouver, siguiendo las normas generales de los Requisitos Uniformes para los Manuscritos presentados a Periódicos Biomédicos (www.ncbi.nlm.nih.gov/books/NBK7256/). Para citaciones en el texto, use números arábigos en sobrescrito,[1] sin espacio, después la palabra y después la puntuación si hubiera: "La descripción de la enfermedad de Parkinson[1] ataca a los idus de 1950,[2] cuando...". En algunos casos, los nombres de los autores pueden aparecer en el texto: "Phillips[12] evaluó diversos cuadros (marcos) de...", y deben citarse en el texto hasta dos autores: "Handel y Matias[15] hicieron un estudio sobre...". Sin embargo, cuando el número de autores es de seis o más, se debe citar el primer autor añadiendo la expresión "y colaboradores": "Silveira y colaboradores[13] propusieron una nueva metodología...".
- **Referencias:** todas las referencias citadas en el texto deberán constar en la lista de referencias. Las referencias deben restringirse a material publicado, artículos o resumes. Los autores son responsables de llenar las referencias de modo preciso y completo. Para referencias con más de un autor, debe enumerarse los nombres por completo de hasta seis autores, por encima de esto, se debe enumerar los seis primeros autores seguidos de "*, et al.*". El total de referencias no debe exceder 40.
- **Tablas y/o figuras:** deberán sumar como máximo cinco.
- **Tabla:** debe elaborarse con programas apropiados, tales como Excel, pueden tener el ancho proporcional al ancho de una página diagramada, teniendo en cuenta el tamaño de fuente Arial 9, espacipo simple. Se deben entregar en archivo de texto: DOC (Microsoft Word), RTF (Rich Text Format) ou ODT (Open Document Text). Numeradas en orden ascendente y acompañadas por título y/o subtítulo explicativo, con una referencia objetiva en el texto. En ningún caso el contenido de una tabla deberá copiar el de una figura o vice-versa. Deben numerarse en orden creciente con números arábigos, conforme aparezcan en el texto.
- **Figuras:** pueden ser fotos, ilustraciones, gráficos, diseños etc. Deben enviarse en archivos separados (formato *tiff ou JPEG). Se deben numerar en orden creciente con números arábigos, conforme su aparición en el texto.

4. Otras sumisiones:

Editorial: Se trata de un comentario y análisis concerniente a un artículo en la edición en cuestión. Puede incluir una figura o tabla y se limita a 750 palabras, de hasta cinco referencias. Será elaborado por el editor y/o algún colaborador invitado por él.

Cartas al editor: espacio de comunicación para los lectores con relación a los artículos recién publicados, limitados a 200 palabras (excluyendo referencias), hasta cinco referencias y 1 figura o tabla, debiendo ser sometida en hasta seis meses después de la publicación del artículo. Para cartas con temas no relacionados con los artículos del **BJHBS** deben limitarse a 500 palabras (excluyendo las referencias), con un máximo de cinco referencias y una figura y/o tabla. Se solicitan los datos de los autores, así como la dirección de correspondencia y/o declaraciones de posibles conflictos de intereses. La decisión de publicar el contenido de la carta es la responsabilidad del editor.

5. Sumisión on-line:

Los artículos y otros tipos de colaboraciones deben ser enviados al correo electrónico submission.bjhbs@hupe.uerj.br, junto con la carta de presentación. El asunto del correo electrónico debe ser: "Tipo de manuscrito [articulo original, reporte de caso, artículo de revisión o carta al editor] – título del manuscrito" + el último apellido del autor principal en MAYÚSCULAS.

Todas las comunicaciones posteriores se llevarán a cabo a través de la opción "responder" de este primer correo electrónico.

El comité editorial valorará el manuscrito de acuerdo con la línea editorial de la revista y responderá con respecto a la aceptación para la valoración por pares en el menor plazo posible. En caso de que sea considerado adecuado para publicación, de acuerdo con la política editorial del **BJHBS**, entrará en el flujo editorial y pasará por las etapas de revisión textual y diagramación.

Después de la aceptación del artículo deberá enviarse la declaración de transferencia de los derechos de autor y declaración de conflictos de interés.

Una prueba de imprenta sera enviada para la valoración final en formato pdf antes de la publicación definitiva del texto y deberá devolverse en el plazo establecido por el equipo editorial.

Los textos y artículos que no cumplan con las especificaciones descritas en estas normas serán devueltos sin previa valoración del consejo de 'editores del **BJHBS**. Esos textos deberán volverse a someter al proceso de valoración.