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SUMMARY

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SECTIONS

ORIGINAL ARTICLES

REVIEW ARTICLES

It is impossible to know the way if we do not know where to start: tidal volume, driving pressure, and positive end-expiratory pressure

É impossível saber o caminho se não soubermos por onde começar: volume corrente, *driving pressure* e pressão expiratória final positiva

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Like many other technologies, mechanical ventilation has emerged out of necessity. Today, it is the basis of cardiopulmonary resuscitation, intensive medicine and anesthesia. Implementation of mechanical ventilation allows the treatment of several diseases that were previously lethal, and increases the survival of thousands of patients every day.

The lungs in acute respiratory distress syndrome (ARDS) are characteristically heterogeneous with an aereted area, called the baby lung, primarily located in non-dependent regions of the lung[1] and areas where there is great inflammation, and the alveoli are filled with residual inflammatory material. It has been described that lungs with ARDS are not hardened but small, and that the compliance of this specific small aerated area is practically normal.[2,3] The complacency of the respiratory system, in turn, is related to the functional size of the lung, that is, the remaining aerated volume.[1]

When this syndrome was described, the mechanical ventilation strategy used high flow volumes (10-15 mL/kg) and relatively low positive end-expiratory pressure (PEEP) (5-15 cmH_2O) with $FiO_2 \leq 0.70$.[4]

Initially, we learned that this ventilatory strategy usually leads to ventilatory-induced lung injury (VILI) because it causes mechanical stress of the lung with overdistension and stretching of the pulmonary parenchyma (volutrauma).[5] We know that VILI also occurs when there is low final expiratory volume. In this case, some bronchioles and alveoli will collapse during exhalation and reopen at the next inspiration, a process that damages the pulmonary parenchyma (atelectrauma) if this takes place repeatedly.[5] Later, it was identified that the forms of mechanical ventilation that cause atelectrauma or volutrauma can lead to the release of inflammatory mediators in the lung, characterizing biotrauma.[5] Together, it is theorized that there may be translocation of inflammatory mediators, bacteria or endotoxins into the systemic circulation due to the increased permeability caused by the underlying disease or biotrauma. This could eventually lead to multiple organ failure.[6-8]

In this context, and because we have not yet been able to demonstrate that a specific mode of mechanical ventilation improves patient survival,[9] one of the major advances in the last decades has been the recognition of its complications, as described above, and the development of ventilatory techniques that minimize these complications.[10]

Ventilatory strategies that reduce the adverse effects of mechanical ventilation and at the same time are associated with better survival are referred to, in all, as protective ventilatory strategies. In general, these strategies involve the use of low tidal volume, low pressure at the end of inspiration (*plateau* pressure), and high PEEP.

Two systematic reviews by The Cochrane Collaboration,[11,12] including six studies on ARDS,[13-18] concluded that protective ventilatory strategies, specifically low tidal volume and low inspiratory *plateau* pressure, reduce hospital mortality and morbidity.

However, distinguishing which element has the greatest value for improving the clinical effects resulting from the protective ventilatory strategy is challenging, since each of them – low tidal volume, low inspiratory pressure (*plateau* pressure), and high positive end-expiratory pressure –, is closely connected to the others.

Therefore, considering that, in ARDS, respiratory system complacency is related to the functional size of the lung, Amato et al.[19] theorized that the ratio between tidal volume and respiratory system complacency, that is, driving pressure, would be an independent risk factor for survival in patients with ARDS.

To prove their theory, Amato et al.[19] conducted a meta-analysis of individual patients with data from 3,562 patients enrolled in nine randomized clinical trials comparing mechanical ventilation strategies in ARDS. The authors' regression model showed that only four variables

were associated with the outcomes; two were related to the patient, APACHE III or SAPS (RR = 1.36) and arterial pH (RR = 0.73), and the other two were related to mechanical ventilation parameters, FiO_2 (RR = 1.24) and driving pressure (OR = 1.42). However, only exposure to high driving pressure during the first days of mechanical ventilation was strongly associated with a fixed and permanent risk over the first 60 days after randomization, thus satisfying the proportional hazards hypothesis. By analyzing the driving pressure more closely, the authors were able to demonstrate that with a fixed PEEP, increased driving pressure leads to increased mortality. On the other hand, when we progressively increase PEEP and maintain the driving pressure fixed, there is no effect on mortality, but if we decrease the driving pressure, we also reduce mortality. Thus, the association of driving pressure with mortality was evidenced. Thus, an association between driving pressure and mortality was evident. Furthermore, in a series of additional analyzes, Amato et al. showed that in patients receiving protective mechanical ventilation (*plateau* pressure ≤ 30 cmH_2O and TV ≤ 7 mL/kg for an ideal weight) a driving pressure of less than 13 cmH_20 generates less mortality, while a *plateau* pressure > or ≤ 26 cmH_20 or TV > or ≤ 7 mL/kg for the ideal body weight has no effect on mortality.

If we consider these new evidences relevant and apply them to the bedside, the main ways of manipulating ventilatory parameters to benefit patients are to reduce tidal volume and adjust PEEP level. The effect of decreasing tidal volume has already been pointed out in the ARDSNet Tidal Volume trial,[20] which showed that the group of patients receiving low tidal volumes had lower mortality. Not coincidentally, this group also had a lower driving pressure, with a difference of -8.8 cmH_2O compared to the group of higher tidal volumes. The three largest randomized controlled trials comparing high versus low PEEP in patients with ARDS failed to demonstrate benefit in relation to the mortality of elevated PEEP. What probably happened was that the changes in driving pressure in these studies[21-23] were not important enough, -2.8, -0.2, +0.2, respectively, to produce a difference in mortality. And this, in turn, may have occurred because in each patient the high PEEP had conflicting consequences: either it reduced VILI by decreasing atelectrauma, or it increased it as a result of overdistension. Given the heterogeneity of the lung with ARDS, "recruitability" is difficult to anticipate, and so adjusting PEEP is of paramount importance.

Thus, the first step is to determine whether the patient has recruitable lungs. Grasso et al. demonstrated that in patients with recruitable lungs, the use of high PEEP causes the PaO_2/FiO_2 ratio to increase and, in contrast, it does not change in patients with non-recruitable lungs.[24] Next, we must determine the optimal PEEP. At this moment, the findings of Amato et al. are of fundamental importance. The adjustment of optimal PEEP can be performed by determining the PEEP resulting in lower driving pressure, preferably less than 14 cmH_2O.[19] This can be done by increasing or decreasing PEEP by 4 cmH_2O at a time, and measuring the driving pressure at each pressure level. Increases or decreases smaller than this can cause problems in the signal/noise ratio. These small additions or decreases in PEEP should be made until the driving pressure reaches its lowest value. If this point is exceeded, the driving pressure increases again. Also in favor of the driving pressure, there is no need for special equipment to measure it, the pressure can be observed directly from the mechanical ventilation device without paralyzing the patient, who only has to be relaxed during exhalation of the gases so that the measured *plateau* pressure is more reliable.

Recently, Gattinoni et al.[25] presented an interesting proposal that energy supplied per unit of time ("power") is an entity that unifies most of the parameters used in mechanical ventilation, in addition to the relevant forces involved in the genesis of VILI, providing us with a "composite index" that can be transposed into clinical practice, as it evaluates the relative contribution of adjustable components at the bedside (TV, frequency, PEEP, Δ airway pressure, I:E ration, flow).

One should emphasize that because ARDS is distinguished by the heterogeneity of pulmonary involvement as well as by conflicting responses to the therapies implemented, such as maintaining high PEEPs, evaluation by means of images of the adequacy of mechanical ventilation is fundamental. The gold standard for mechanical ventilation monitoring in ARDS, however, is computed tomography, despite its disadvantages due to risks related to the transport of patients and their excessive exposure to radiation, which reduces its applicability.[1] On the other hand, there is electrical impedance tomography, which seems to be a very appropriate alternative, providing continuous monitoring in real time, without the need for radiation or patient transport. This test reliably shows changes in lung volume and tidal volume.[1,26]

The strategies of protective ventilation are effectively a breakthrough. They allow the reduction of mortality despite the partial understanding of how this benefit is achieved. The additional understanding provided by Amato et al.[19] solved an old issue while simultaneously providing key elements for reducing mortality and allowing us to establish a strategy for optimum PEEP adjustment, friendly to the professionals and which does not require special equipment.

Mechanical ventilation in ARDS remains a major challenge for intensive care physicians, but newly aggregated knowledge and the new technologies available open a new perspective on the path that will still be pursued.

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What is the role of routine ultrasonography performed in the first trimester of low-risk pregnancy?

Qual é o papel da ultrassonografia de rotina realizada no primeiro trimestre em gestações de baixo risco?

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Final draft: April 2016

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The Guidelines Project, an initiative of the Brazilian Medical Association, aims to combine information from the medical field in order to standardize procedures to assist the reasoning and decision-making of doctors.
The information provided through this project must be assessed and criticized by the physician responsible for the conduct that will be adopted, depending on the conditions and the clinical status of each patient.

Structured question

The clinical question is structured according to the PICO components: P (Patient), I (Intervention), C (Comparison), O (Outcome).

- **P:** Low-risk pregnancy (first trimester)
- **I:** Ultrasonography
- **C:**
- **O:**

Bases of scientific data consulted

The scientific database searched was PubMed-Medline. A manual search of the references of reviews (narrative or systematic) was also performed.

Strategies for search of evidence

PubMed-Medline
Strategy: (Pregnancy Trimester, First OR First Trimester OR Phases, Early Placental OR Pregnancy Trimesters, First OR First Pregnancy Trimester OR Pregnancies, First Trimester) AND (Ultrasonography OR Echography OR Ultrasonic Diagnoses). N=838

Results of the evidence selected

The evidence was assessed according to the Oxford classification, which establishes the strength of the evidence based on the study design chosen.

Discussion

Ultrasonography allows the evaluation of the fetal morphology and biometric data, the examination of the fetal anatomy and the detection of major congenital defects, as well as subtle markers that signal the possibility of chromosomal abnormalities and genetic syndromes. Until a few years ago, early ultrasonography aimed to identify the number of fetuses, to verify chorionicity in the case of multiple pregnancies, and to date the pregnancy. In recent years, however, with improved technology providing images with better resolution, as well as access to ultrasound examinations for a greater number of pregnant women, early identification of embryo-fetal defects has been favored. The objective of this review was to evaluate the contribution of ultrasound performed in the first trimester (between weeks 11 and 14) in low-risk pregnancies (except in the situations listed in Table 1). Aspects assessed include determining gestational age, characterizing multiple pregnancies, and evaluating the fetal anatomy. With regard to the diagnosis of malformations, it is possible to detect structural abnormalities and genetic syndromes.

Verification of gestational age

Calculating gestational age continues to be an extremely important measure in prenatal follow-up and reproductive research, allowing appropriate assessment of fetal development. Inaccuracies are inherent to the ultrasound imaging method and often observed, especially measurement errors and the biological variability of the fetus. Numerous studies have revealed that the estimated gestational age obtained from ultrasound proved to be superior to that obtained using the date of the first day of the last menstrual cycle (LMP), predicting even more safely the probable date of delivery.[2-4] (**B**) This fact stems mainly from the difficulty many women have in inform-

TABLE 1 Risk factors that may indicate referral to high-risk prenatal care.

Factors related to previous conditions
Heart diseases, severe lung diseases (including bronchial asthma), severe kidney diseases (such as chronic renal failure and transplant patients), endocrine diseases (particularly diabetes mellitus, hypothyroidism, and hyperthyroidism), hematological disorders (including sickle cell disease and thalassemia), chronic hypertension and/or patients treated with antihypertensive medication (PA > 140/90 mmHg before gestational age of 20 weeks), neurological diseases (such as epilepsy), psychiatric disorders requiring monitoring (psychosis, severe depression, etc.), autoimmune diseases (systemic lupus erythematosus, other collagenoses), maternal genetic diseases, history of deep venous thrombosis or pulmonary embolism, gynecological disorders (uterine malformation, myomatosis, adnexal tumors, and others), patients with infectious diseases such as hepatitis, toxoplasmosis, HIV infection, tertiary syphilis (USG with fetal malformation), and other STDs (condyloma), Hansen's disease, tuberculosis, licit or illicit drug addiction, any clinical pathology that requires specialized monitoring.
Factors related to previous reproductive history
Intrauterine or perinatal death in previous gestation, especially if the cause is unknown; previous history of hypertensive gestational disease with poor obstetric and/or perinatal outcome (premature termination of pregnancy, intrauterine fetal death, Hellp syndrome, eclampsia, maternal ICU admission); repeated abortion; infertility.
Factors related to the current pregnancy
Restriction of intrauterine growth; polyhydramnios or oligohydramnios; twin pregnancy; fetal malformations or fetal arrhythmia; hypertensive disorders of gestation (pre-existing chronic hypertension, gestational or transient hypertension); recurrent urinary tract infection or two or more episodes of pyelonephritis; severe or unresponsive anemia after 30-60 days of treatment with ferrous sulfate; patients with infectious diseases such as hepatitis, toxoplasmosis, HIV infection, tertiary syphilis (USG with fetal malformation), and other STDs (condyloma); infections such as rubella and cytomegalovirus acquired in the current gestation; laboratory evidence of proteinuria; gestational diabetes mellitus; severe maternal malnutrition; morbid obesity or low weight.

Adapted from: Ministério da Saúde, 2012.[1]

ing the exact date of the last menstrual period or the misinterpretation of genital bleeding related to egg implantation as menstrual bleeding. In addition, the convention of a 14-day interval between menstruation and ovulation may render gestational age calculations inaccurate, especially for women with irregular menstrual cycles.[5] (**B**)

Finally, studies have consistently shown that the use of the date of the last menstrual period for the purpose of estimating gestational age is related to a greater frequency of a later due date compared to that which is verified when this estimate is obtained from the early ultrasound (10 to 12% versus 4%, respectively).[3,6] (**B**)

Early detection of a multiple gestation

It is known that multiple pregnancies are associated with increased perinatal morbidity and mortality compared to single fetus pregnancies. Therefore, early identification of multiple pregnancies and determination of the type of placenta play an important role in the risk stratification and monitoring of twin pregnancies, contributing significantly to a better prognosis. Observational studies designed to analyze the accuracy of ultrasonography performed during the first trimester in multiple pregnancies to predict chorionicity consistently found high values of sensitivity, specificity, positive and negative predictive values compared to those observed from ultrasonographies performed in the second trimester.[7,8] (**B**) Thus, ultrasound, especially when performed by the end of week 14, is a reliable tool for determining the number of chorions in a twin pregnancy.

Evaluation of fetal morphology

First trimester ultrasound, performed between week 11 and week 14 of pregnancy, aims to track chromosomal abnormalities, genetic syndromes and other fetal malformations. This imaging method is well-established for the screening of aneuploidies. In 2008, Kagan et al. showed that the increase in cutaneous thickness present in individuals with Down syndrome could be seen in the first trimester (from 11 to 13 weeks plus 6 days). They also verified that increased nuchal translucency (thickness above the 95th percentile for gestational age), when associated with maternal age and biochemical tests such as maternal serum levels of beta-hCG free fraction and pregnancy-associated plasma protein (PAPP-A) provided a detection rate of 90% of cases of trisomy 21 with a false-positive rate of 3%.[9] (**B**) Even in the absence of chromosomal abnormality, fetuses exhibiting increased nuchal translucency have an increased risk of intrauterine death and structural abnormalities, especially cardiac.[10] (**B**)

Some abnormalities like anencephaly are almost always detected. However, others such as myelomeningocele or microcephaly may be difficult or impossible to identify. Numerous studies analyzing the performance of first trimester ultrasonography to detect fetal abnormalities found that the findings were either incidental during screening for aneuploidy or were detected after careful

examination of euploid fetuses due to increased nuchal translucency.[11] (**D**) In this regard, the findings of the related studies do not reflect the true performance of first-trimester ultrasonography for the screening of nonchromosomal abnormalities. A multicenter clinical trial conducted by Saltvedt et al. was designed to analyze the sensitivity of morphological ultrasound examinations performed during the first and second trimesters to identify fetal malformations. To do so, they randomized more than 39,000 pregnant women to perform a single ultrasound during pregnancy, between weeks 12 and 14, or between weeks 15 and 22. The authors found a detection rate of 38% for the first group compared to 47% for the second, but the difference was not significant.[12] (**B**)

Recommendation

Routine ultrasonography performed in the first trimester of low-risk pregnancies allows a better calculation of gestational age, preventing diagnosis of a later due date and consequent iatrogenic preterm delivery. It also allows the characterization of the number of fetuses and chorionicity.[13] (**A**). This imaging modality also contributes to the identification of aneuploidy and, increasingly, to the diagnosis of structural anomalies.

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Sensorineural hearing loss as the first manifestation of Sjögren's syndrome

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Introduction

Dysfunction of inner ear, vestibulocochlear nerve or central brain processing centers leads to sensorineural hearing loss (SNHL). Autoimmune inner ear disease (AIED) is a rare but potentially treatable cause of hearing loss, characterized by progressive evolution over weeks to months.[1] In 1958, an antigen-antibody reaction was described in 13 patients with progressive bilateral SNHL,[2] and later, in 1979, the term autoimmune SNHL was proposed after an idiopathic bilateral progressive SNHL had markedly improved with steroids therapy.[3] Since there are no defined diagnostic criteria for AIED, it remains a diagnosis of exclusion, supported by clinical suspicion and responsiveness to corticotherapy. About one third of the cases of AIED is secondary to a systemic autoimmune disease and may, rarely, be the first manifestation of the latter.[4,5]

Method

Description of a clinical case, based on the data referred to in the clinical process.

Results

We present the case of a 65-year-old woman who attended the otorhinolaryngologist due to progressive and cumulative unilateral left hearing loss over one month, without any other accompanying symptoms. She had been diagnosed with breast cancer eleven years before, being successfully treated with chemotherapy and right mastectomy. She was medicated for arterial hypertension and hypothyroidism. Her son had been recently diagnosed with a cerebellopontine angle tumor. She had no history of vertigo or ocular problems. Otoscopy was normal but the Weber test lateralized to the right and the Rinner test were both positive. The neurological examination was normal, including no cerebellar or vestibular findings. The remaining physical test was unremarkable.

An audiogram was performed, with left hearing loss described as -70 dB in the 8000 Hz range, confirming the suspicion of SNHL (Figure 1A). The routine laboratory workup and magnetic resonance imaging were normal.

She was started on 60 mg of prednisolone (1 mg/kg/day) with immediate response (Figure 1B). Steroid tapering was started and, after four weeks, she stopped the treatment. A relapse of symptoms occurred during the following month, with deterioration of hearing levels registered in the audiogram (Figure 1C). Corticotherapy was restarted and AIED was considered the most likely diagnosis, therefore she was referred to the autoimmune diseases clinic to exclude a systemic autoimmune disease.

She complained of dry eyes and mouth but denied arthralgia, parotid swelling or cold extremities, and had no history of recurrent abortion. The physical examination remained normal aside from mucosal dryness confirmed by the Schirmer test (< 5 mm in 5 minutes). Laboratory tests showed normal liver and thyroid function, with positive thyroid peroxidase antibodies. Antinuclear antibodies were positive – she had anti-mitochondrial M2 antibodies but anti-Ro and anti-La where negative. Other autoimmune tests, including rheumatoid factor, anti-neutrophil cytoplasmic, anti-centromere, anti-neuronal antibodies, and lupus anticoagulant, were negative. She was observed by an ophthalmologist who reported no significant findings. The thyroid echography showed a microgranular pattern. She was treated with pilocarpine and a scintigraphy was performed, showing hypofunction of the salivary glands. The biopsy revealed significant lymphoid infiltrate – class III on the Chisholm Mason scale – confirming Sjögren's syndrome (SS).

After four months of low dose corticotherapy (5 mg of prednisolone), she had no symptoms of hearing loss and her left audiogram showed a 50 dB improvement (Figure 1D).

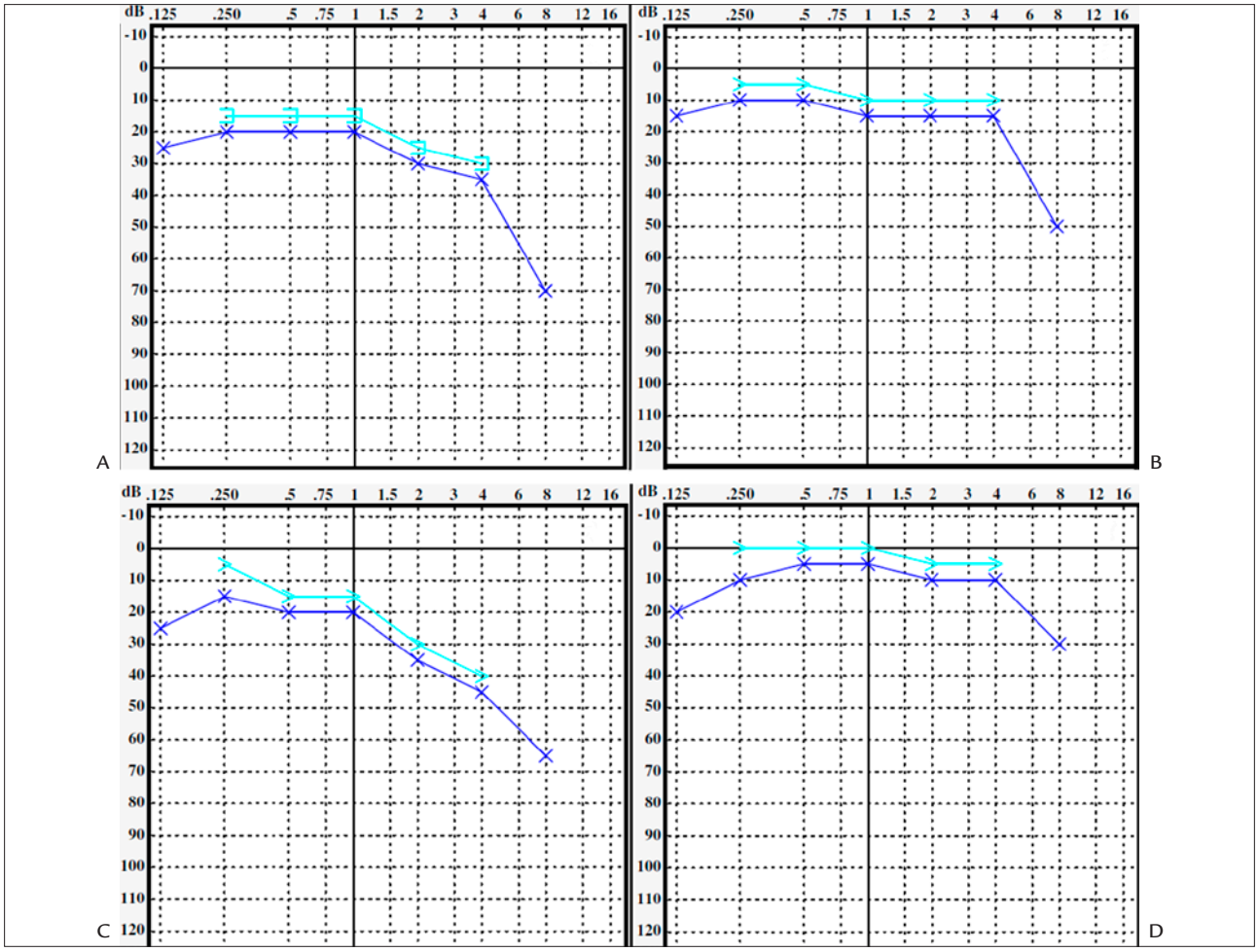

FIGURE 1 A. First audiogram, with left hearing loss described as -70 dB in the 8000 Hz range, confirming the suspicion of SNHL. B. Audiogram on 60 mg of prednisolone. C. Audiogram after stopping steroids, showing deterioration of hearing levels. D. Audiogram after four months of low dose corticotherapy, showing a 50 dB improvement.

Discussion

AIED is a SNHL which may have uni- or bilateral involvement and presents with progressive deterioration of auditory function over weeks to months. The time course is very important for the differential diagnosis between sudden hearing loss (hours to days) and presbycusis (years). It usually affects people in between the third and the sixth decade of life. Vestibular symptoms may be present.[3,5,6]

SNHL can be the initial manifestation of a systemic autoimmune disease such as SS, systemic lupus erythematosus, granulomatosis with polyangiitis (Wegener's),[7] polyarteritis nodosa, rheumatoid arthritis, and Cogan's syndrome.[8,9] There is a significant association between thyroid autoimmunity and Ménière's disease, which can itself be very difficult to distinguish from AIED in the first months,[10] but our patient had no vestibular symptoms. The relationship between SS and AIED was studied by Tumiati et al., who suggested the performance of audiometric tests in patients with this syndrome.[11] In this case, despite seronegative anti-Ro and anti-La, there were clinical, imaging, and histological findings that allowed the diagnosis of SS. Although anti-mitochondrial antibodies are characteristic of primary biliary cirrhosis, it is known that their presence in SS patients can occur, presenting a higher risk of liver involvement,[12] which increases the importance of surveillance by a multidisciplinary team. Systemic corticosteroids should be initiated as soon as possible since the prognosis is time dependent.[5]

Conclusion

This case, in which the medical findings led to the diagnosis of SS, illustrates the importance of a multidisciplinary approach and clinical suspicion of an autoimmune

cause for progressive SNHL. After a response to corticotherapy, the possibility of an association with autoimmune systemic diseases should be thoroughly investigated.

Key messages

- Autoimmune inner ear disease is a diagnosis of exclusion, supported by clinical suspicion and responsiveness to corticosteroids.
- It is secondary to an autoimmune disease in one third of the cases.
- Although it is an unusual etiology of hearing impairment, it is important to recognize it, as early diagnosis and treatment can have a marked effect on the clinical outcome.

Notes

- No funding to declare.
- No animal or human studies were carried out by the authors for this article.
- The authors declare that none of the presented data allows for the identification of the patient.

Conflict of interest

The authors declare no conflict of interest.

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Synthetic cannabinoids in the kidneys

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Summary

Acute kidney injury is an important cause of mortality and morbidity today and can occur due to several reasons. As time, geographic regions, and living conditions change, various etiological agents arise with nephrotoxic effects. Awareness of such nephrotoxic effects has been raised with the increasing frequency of addictive substance use, especially among young people in society.

Keywords: synthetic cannabinoids, acute kidney injury, toxicity.

Introduction

Synthetic cannabinoids (SC) are addictive substances with an increasing frequency of use among the young population. It is reported that the rate of synthetic cannabinoid use among United States (US) high school students is approximately 11 to 12%.[1] The detection of these substances in plasma and urine samples by toxicology tests has some difficulties because producers frequently change the molecular structure to avoid breaking the law. This "ghost" effect and the relatively cheap price compared with other addictive substances make SCs popular among this population.

These compounds have several psychiatric, metabolic, and physiological effects. In recent years, cases of acute kidney injury (AKI) have appeared due to the use of cannabinoids. Most of these cases show complete improvement without biopsy and, therefore, biopsy findings are known in a very limited number of such cases.

Although the general histopathological finding is consistent with acute tubular necrosis (ATN), there are also cases with detected findings of tubulointerstitial nephritis. It is important to enhance awareness in this regard and also to emphasize the likelihood of developing renal injury in addicted patients.

What are synthetic cannabinoids?

Synthetic cannabinoids are marijuana-like, non-natural, chemically produced components. Their molecular structure shows much similarity with Δ9-tetrahydrocannabinol (THC), the main active compound in marijuana. Although they are similar, SCs are more potent molecules. They act like agonists for cannabinoid (CB) receptors. Cannabis-like (mimetic effect) components in SCs affect cell receptors (CB1 and CB2) in the brain, causing an effect 100 times more potent than THC contained in normal marijuana. SCs may not be as "innocent" as marijuana and have more potent and different side effects due to their biochemical differences. Some of these components are HU-210, CP 47, 497, JWH-018, JWH-073, JWH-398, and JWH- 250.

SCs are a group of substances with addictive and psychoactive effects, which is highly popular especially among young people due to its cannabis-like effects. The commercial products are sold under the name "k2," "spice," and "black mamba" in the US and the European Union (EU), and "Bonsai" and "Jamaica" in Turkey.

Some other well-known products containing SCs are "spice gold," "spice silver," "spice diamond," "silver," "yucatan fire," "sence," "chill X," "smoke," "gnie," "algerian blend." The formal reports both from the US and EU indicate that the rate of SC use is growing faster every year. They can cause many health problems, from acute severe crises to several organ dysfunctions (arrhythmia, anxiety, agitation, confusion, hypertension, seizures, hallucinations, AKI, and myocardial infarction) and even death.[2]

Synthetic cannabinoid-related acute kidney injury (SC-RAKI)

In recent years, renal injury-causing effects of SCs have been encountered, even though they are rare. The most common

result of renal exposure is AKI. A study from the US found renal injury of approximately 0.9% due to SC use.[3]

The mechanism of renal injury is not yet clearly defined.[4] Acute tubular necrosis seems to be the leading etiology for AKI among these patients. However, classical hemodynamic collapse, hypotension or hypovolemic status for development of ATN may not be present. Cardiac effects of SCs are also very well-described. The negative inotropic effects on hemodynamic processes may have impact on renal blood flow decrease. These cardiac effects also may aggravate prerenal conditions. Rhabdomyolysis and cannabinoid hyperemesis syndrome are the second most commonly seen etiologies.[5] Also, direct toxic effects of the SC molecules or addictive substances during elimination from the kidney may be responsible. Unfortunately, it is not clear which components are responsible for their nephrotoxic effect. Some reports revealed that in particular XLR11 and UR-144 N-pentanoic acid metabolite were detected in the serum and urine samples of the cases with SC-RAKI.[6] An experimental study of Barutta et al. showed that blockade of CB1 receptors has been shown to have protective effects on renal function and ameliorate albuminuria in diabetic mice.[7]

As we have a look at the literature, obviously, renal biopsy for SC-RAKI is not much preferred. The "prerenal" nature of AKI in these patients might have caused this approach. The renal biopsy applied cases mostly revealed ATN. To a rare extent, tubulointerstitial nephritis and crescentic glomerulonephritis were reported (Table 1).

Additionally, hypokalemia and hypertension may occur during SC use, but less frequently.[8]

Reported etiologies for SC-RAKI

Cannabinoid hyperemesis syndrome (CHS)

Cannabis is known for its anti-emetic effects; however, SCs may cause intractable nausea and vomiting. The most common symptoms of the patients at hospital admission are nausea and vomiting due to SC use and those were consistent with ATN in kidney biopsies. This clinical situation is called as cannabinoid hyperemesis syndrome (CHS). Allen et al. described this entity first in 2004.[9] In some papers "hyperemetic hydrophilic syndrome" is also used as synonym.

This syndrome is more common in chronic users and courses with periodic vomiting (especially in the morning), abdominal pain or discomfort, and the desire for a hot shower. Long-term use of SCs is essential in provoking symptoms. The cyclic pattern of these symptoms is pathognomonic. Prerenal kidney injury may occur secondary to this presentation.[10,11] Acute tubular necrosis is the most common reason causing renal injury so biopsy may not be indicated if the clinical presentation is as stated.[12] A diagnostic algorithm was described for such patients.[13] The treatment approaches are palliative. Satisfactory results with haloperidol, intravenous NaCl 0.9%, paracetamol, lorazepam, ondansetron, and morphine use are reported in the literature.[14,15]

Rhabdomyolysis

Rhabdomyolysis was also shown to cause kidney injury in these cases. However, it is not clear whether direct toxic effects of compounds in SCs on muscles are responsible for this entity. Some other side effects of SCs like hypokalemia or hyperthermia may also have contribution.[16-18] In addition to these two clinical approaches, unfortunately the pathophysiology of cannabinoid-associated renal injury has not been fully clarified. The treatment strategies for SC induced rhabdomyolysis did not differ from classical approaches.

Tubulointerstitial nephritis

With respect to cannabinoid-associated kidney injury, the literature very rarely contains cases with acute tubulointerstitial nephritis, as well.[19] Although acute tubulointerstitial nephritis may be directly caused by the effect of SC, various nephrotoxic chemical substances or heavy metals added to these synthetic compounds that have no standardization may also have an effect on such biopsy results.[20] There are very few reports of the co-existence of SC-RAKI and tubulointerstitial nephritis in the literature.[19,21] In some cases tubular oxalate crystals accompanying tubulointerstitial nephritis were described.[22] There is no specific treatment for cannabinoid-associated effects. Support therapy, cardiac monitoring, and follow-up of consciousness and vital signs are essential.

Conclusion

One of the current, most socially important and growing problems is the increasingly frequent use of synthetic cannabinoids and similar substances, especially among the young population. Addicts using such substances may present not only with central side effects such as psychiatric symptoms, seizure, mania, and changes in consciousness, but also with renal dysfunction. Synthetic substance use should be considered in patients who are young, without any chronic diseases, and with AKI that may present with cognitive and psychiatric symptoms. Failure to identify all components in routine urine toxicological analyses can lead to misdiagnosis. Monitoring kidney function tests is important during the follow-up of patients since SCs have the potential to increase the occurrence of acute kidney injury.

TABLE 1 Recently reported cases of SC-RAKI (between 2013 and 2016).

Author	Age	Peak creatinine (mg/dL)	Peak creatinine kinase (U/L)	RRT	Renal biopsy	Renal pathology	ESRD
Sweeney et al.[16]	27	2.2	57,050	No	No	Rhabdomyolysis	No
Argamany et al.[17]	27	13.88	> 40,000	No	No	Rhabdomyolysis	No
Zhao et al.[18]	39	9.7	148,643	HD	No	Rhabdomyolysis	No
Elçioğlu et al.[12]	28	6.5	191	No	Yes	Acute tubular necrosis	No
Ukaigwe et al.[11]	38	4.78	??	No	No	Prerenal (CHS)	No
Gudsoorkar et al.[4]	26	8.1	2,337	HD	No	Not specified	No
Sherpa et al.[5]	45	3.06	301,901	CRRT	No	Rhabdomyolysis	No
Kamel et al.[22]	65	5.6	Normal	HD	Yes	TIN, calcium oxalate crystals	?
Kazory et al.[21]	22	7.05	338	No	Yes	Acute tubular necrosis	No
Habboushe et al.[23]	26	3.21	Not specified	No	No	CHS	No
Thornton et al.[24]	26	7.74	Normal	No	Yes	Rare globally sclerotic glomeruli, no evidence of acute glomerular disease	No

HD: hemodialysis; CRRT: continuous renal replacement therapy; CHS: cannabinoid hyperemesis syndrome; RRT: renal replacement therapy; TIN: tubulointerstitial nephritis; SC-RAKI: synthetic cannabinoid-related acute kidney injury; ESRD: end-stage renal disease.

Conflict of interest

The authors declare no conflict of interest.

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Alert for bone alterations and low serum concentrations of vitamin D in patients with intestinal inflammatory disease

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Summary

Background: Inflammatory bowel diseases (IBD), including Crohn's disease (CD) and ulcerative colitis (UC), are characterized by chronic inflammation of the intestine that can reduce the absorption of nutrients such as vitamin D and calcium.
Objective: To investigate bone alterations and serum levels of vitamin D in patients with IBD.
Method: This was a cross-sectional study based on a review of medical records of patients from a private office in Curitiba, PR, Brazil. Serum levels of vitamin D and bone densitometry were measured at diagnosis of IBD. A total of 105 patients were included; 38 (58.4%) with CD; 27 (41.6%) with UC and 40 with irritable bowel syndrome (IBS) as comparison group.
Results: When compared to patients with UC, CD patients showed a higher prevalence of bone alterations, being 15.8% with osteoporosis and 36.8% with osteopenia. In UC, bone alterations occurred in 29.6% of cases, 3.7% with osteoporosis and 25.9% with osteopenia. As for vitamin D levels, among CD patients, 10.5% had vitamin deficiency, 65.8% insufficiency and 23.7% were sufficient. In UC, 7.4% of cases had deficiency, 74.1% insufficiency and 18.5% had sufficient serum levels of vitamin D. In the group with IBS, deficiency was observed in 17.5% of cases, insufficiency in 55% and sufficiency in 27.5% of them. There was no significant difference between groups.
Conclusion: IBD patients have a high prevalence of bone changes, especially those with CD. Serum levels of vitamin D are below the recommended in all the evaluated groups.

Keywords: inflammatory bowel disease, osteoporosis, vitamin D.

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Introduction

Inflammatory bowel disease (IBD) is a term used to refer to Crohn's disease (CD) and ulcerative colitis (UC), characterized by chronic and recurrent inflammation of the bowel. Patients with IBD may present a decrease in bone mineral density (BMD) over the years due to absorptive changes. Osteoporosis is a systemic disease characterized by a decline in bone mass and deterioration of the micro-architecture of the bone tissue. This decline leads to an increase in bone fragility and risk of fractures, which may imply an increase in morbidity poorer quality of life for the patient. Vitamin D (1.25-dihydroxyvitamin D) is the major steroid hormone involved in the regulation of calcium homeostasis and bone metabolism. It can be obtained from foods or by endogenous conversion, depending on the exposure of the skin to ultraviolet rays.

The occurrence of bone changes in patients with IBD is not uncommon, although there is no established protocol to indicate this investigation, especially in young patients. Low BMD in this group of patients is more frequent than in the general population[1] and factors such as chronic inflammation, prolonged use of corticosteroids, unsatisfactory physical activity, and malnutrition may contribute to the appearance and aggravation of the condition.[2,3] In addition, the absorptive changes that patients with IBD present may cause vitamin D absorption deficits. In turn, a serum reduction of this vitamin may contribute to the early onset of bone changes such as osteopenia and osteoporosis.

The present study aimed to investigate bone alterations and serum levels of vitamin D in patients with IBD.

Method

This is a cross-sectional analytical study approved by the Research Ethics Committee of the Sociedade Evangélica Beneficente de Curitiba (PR), under Opinion Report number 726.050. It was based on the review of medical records of patients followed in a private practice in Curitiba, PR. All consultations and procedures were done by the same professional.

The study included patients with the diagnosis of IBD who had the following information presented in their medical records: serum concentration of vitamin D and results of bone densitometry in the lumbar spine and femur using dual-energy X-ray absorptiometry (DEXA). Incomplete medical records were excluded. Of 120 records analyzed, 15 were excluded because they did not meet the inclusion criteria, resulting in 105 for the study. The patients studied were divided into groups with IBD and a comparison group with irritable bowel syndrome (IBS), as follows:

- CD group: n=38, median age of 39.5 years (range 16 to 73 years), of which 22 (57.9%) were female.
- UC group: n=27, median age of 39 years (range 16 to 70 years), of which 21 (77.7%) were female.
- IBS group: n=40, analyzed as comparison group, median age of 52 years (between 21 and 77 years), 31 (77.5%) were female.

Serum levels of vitamin D and bone densitometry had already been investigated at the time of data collection, which occurred between October 2014 and July 2015. Data collection protocols included: sex, age, disease duration, age at diagnosis, smoking status, history and site of low impact fracture, comorbidities, first bone densitometry report, medications being used until the date of the examination, serum vitamin D dosage.

All the tests were done in the same laboratory of clinical analyses. Based on the recommended reference values, serum vitamin D levels below 20 ng/mL were considered deficient, between 20 and 30 ng/mL were insufficient, and above 30 ng/mL, sufficient.

The assessment for BMD was made using DEXA, which was used to estimate BMD at the lumbar spine and femur. In this method, an X-ray tube and detector are used to scan over the area of interest and generate an image of bone mineral content expressed in grams of calcium. BMD is calculated by dividing the bone mineral content by the bone area. This value was compared with that of the BMD of the healthy young population and the result was expressed as T score. According to the World Health Organization recommendation, the criteria used to define BMD were: normal (T score > -1), osteopenia (T score -1 to -2.5), osteoporosis (T score < -2.5), and severe osteoporosis (fragility fractures, T score < -2.5).

Statistical analysis

The data were prepared in Excel (Microsoft, Office, 2013), organized and analyzed statistically with the aid of the GraphPrism 5.0 package, using the appropriate tests. Fisher's exact tests were used for categorical variables and the Kruskal-Wallis test was used to compare vitamin D dosages, analyzing the three groups under study. A significance of 5% ($p<0.05$) was adopted.

Results

Table 1 shows clinical and demographic data of the patients. The median disease duration of patients with IBD was 11 years (range 1 to 41 years) and 16 years (2 to 36 years) for CD and UC, respectively. There was no significant association between disease duration and the presence of low BMD.

Among the CD group, low BMD was observed in 20 patients (52.6%), with osteoporosis in six cases (15.8%) and osteopenia in 14 (36.8%). In the UC group, bone changes were observed in eight cases (29.6%), osteoporosis in one (3.7%), and osteopenia in seven (25.9%). Comparing the two groups, p=0.07 was obtained. Attention was drawn to the occurrence of bone changes in very young patients. Two patients (one male and one female) with CD had a diagnosis of osteoporosis as soon as at 14 years of age. Patients with IBS did not undergo bone densitometry because there was no indication for such procedure.

As for smoking habit, nine (23.7%) of the patients with CD were smokers, as well as six (22.2%) in the group with UC. Among the nine smokers with CD, six were women: two had a history of fracture (one of tibia and one of knee), both had osteopenia on DEXA. Of the six smokers in the group with UC, four were women and one of them had a history of rib fracture and osteopenia. In addition to these patients, in eight other cases (non-smokers), low-impact fractures were observed, with five patients with CD and four women. Three of these women had osteopenia and had had distal metaphysis fractures on the radius and styloid process, ulna, arm, femoral neck, and shoulder. The other patient had osteoporosis and had fractured the humerus. The only man with fracture had a metacarpal lesion and normal bone densitometry. The remaining three patients were women

TABLE 1 Clinical and demographic data of the patients studied.

	Crohn's disease (n=38)	Ulcerative colitis (n=27)	Irritable bowel syndrome (n=40)
Gender			
Female	22 (57.9%)	21 (77.7%)	31 (77.5%)
Male	16	6	9
Age			
Median age (years)	39.5	39	52
Interval (years)	16 to 73	16 to 70	21 to 77
Disease duration			
Median (years)	11	16	4
Interval (years)	1 to 41	2 to 36	1 to 23
Smokers	9 (23.7%)	6 (22.2%)	9 (22.5%)

with UC: two with normal bone densitometry and both had fractured the foot. In addition, one of them had fractured the hand, the elbow and the knee, thus being diagnosed with osteoporosis. There were no cases of fractures among patients in the comparison group.

Data on serum vitamin D concentrations in the groups studied are shown in Table 2. No direct correlation was found between age and serum vitamin D concentration. In the CD group, the median concentrations were 28.7 ng/mL (range 8 to 70.7 ng/mL), with four (10.5%) cases of deficiency, 25 (65.8%) of insufficiency, and only nine (23.7%) patients had sufficient serum levels. In the group of patients with UC, the median was at 30 ng/mL (range 11 to 76.7 ng/mL). Vitamin D deficiency was found in two (7.4%) cases and insufficiency was found in 20 (74.1%); five (18.5%) had sufficient serum concentrations. In the group with IBS, the median was at 26.6 ng/mL (range 11 to 70.5 ng/mL). There was no significant difference in serum vitamin D concentrations between the groups studied.

Discussion

Patients with IBD may have a number of complications, including bone changes that may cause significant clinical repercussions. In our population, there is little research on the presence of these alterations in this group of patients.[8]

Our study found a higher female prevalence for both IBDs, as observed in other Brazilian studies,[4-6] namely 57.9% women with CD and 77.7% with UC. In agreement with the literature,[7] a higher incidence of IBD was found among young adults, with medians of 39.5 and 39 years for CD and UC, respectively. Regarding age, we observed a high prevalence of low BMD in patients with IBD much earlier than observed in the general population.

Osteoporosis is typically a multifactorial disease. It is most often primary in postmenopausal women and in older people. Secondarily, it may be due to prolonged corticosteroid therapy, nutritional changes such as poor calcium intake, vitamin D deficiency, alcoholism, and smoking.[9-12] In our study, we observed that, in women with IBD, fracture cases were more frequent among smokers, a factor already pointed out in another study,[9] which associated smoking with reduction of BMD and with increased risk of fractures.

Souza et al. described that the number of patients with low BMD between the two groups of IBD is equivalent.[3] In our study, however, we observed that CD patients had a higher prevalence of bone alterations (52.6% CD and 29.6% UC, p=0.07). This difference may be related to the fact that in CD a greater area of the digestive tract implied in the absorption of nutrients is affected, or even because these patients use corticosteroids more frequently compared with patients with UC. In addition to IBD, other chronic diseases have been associated with osteoporosis, such as celiac disease, systemic lupus erythematosus (SLE), and cystic fibrosis. Silva et al.[13] showed that 68.3% of the celiac patients at the time of diagnosis had low BMD, 47% had osteopenia, and 32% had osteoporosis. Studying patients with SLE, Bultink[14] described the occurrence of bone changes in 68% of them. As for cystic fibrosis, another study[15] revealed a prevalence of 40 to 70% of bone alterations in adult patients.

In our study, 13 patients with CD (five women and eight men) and three with UC (all women) used corticosteroids, a risk factor for the occurrence of bone mass changes.[1,12,15] Two of the female patients with CD with a history of fracture were perimenopausal, another contributing factor to bone loss.[9,10] Among the patients with UC, only one was menopaused and presented normal BMD.

This study is a warning about the importance of investigating bone changes in all patients with IBD, regardless of the age at diagnosis. As an example, our study includes two patients with CD aged 14 years, treated with corticosteroids and who already showed osteoporosis in their exams.

TABLE 2 Bone changes and serum concentrations of vitamin D in the patients studied.

	Crohn's disease (n=38)	Ulcerative colitis (n=27)	Irritable bowel syndrome (n=40)
Bone densitometry			
Presence of bone changes*	52.6% (20/38)	29.6% (8/27)	NP
Osteoporosis	15.8% (6/38)	3.7% (1/27)	NP
Osteopenia	36.8% (14/38)	25.9% (7/27)	NP
Vitamin D			
Median concentration**	28.7	30.0	26.6
(min – max, ng/mL)	(8 – 70.7)	(11 – 76.7)	(11 – 70.5)
Deficient (< 20 ng/mL)	10.5% (4/38)	7.4% (2/27)	17.5% (7/40)
Insufficient (20 to 30 ng/mL)	65.8% (25/38)	74.1% (20/27)	55% (22/40)
Sufficient (> 30 ng/mL)	23.7% (9/38)	18.5% (5/27)	27.5% (11/40)

NP: not performed.
*p=0.07, Fisher's exact test comparing CD and UC.
**p=0.40, Kruskal-Wallis H test.

Vitamin D deficiency can occur due to intestinal absorption impaired by diseases such as IBD. However, it is known to be multifactorial, being influenced by factors such as lack of sun exposure, low intake of foods rich in vitamin D, prolonged glucocorticoid therapy, senility or hormonal changes triggered by menopause. The three groups in our study had inadequate serum levels of vitamin D, and even in the comparison group with IBS, 55% of the patients showed insufficient concentrations. Premaor et al.[16] reported that serum vitamin D levels in both young and old adults varied by geographic region, depending on latitude, and were more appropriate near the equator. In the Curitiba area, due to its climatic characteristic of low annual insolation, there is a greater possibility of vitamin D deficiency, as already evaluated by other authors.[3,16] Regarding serum vitamin D levels, the importance of its replacement in patients with IBD has already been suggested,[17] since it has a relevant role in the health of this group of patients. In addition to the benefit in bone integrity, an improvement in the immune response is expected and, although not yet fully proven, aid in the prevention of colorectal cancer, a complication that can occur in patients with IBD.[17,18]

We conclude that our patients with IBD had a high prevalence of bone alterations, found to a greater extent in those with Crohn's disease. Additionally, we observed that serum levels of vitamin D were below the recommended levels in all groups studied.

Conflict of interest

The authors declare no conflict of interest.

Resumo

Alerta para alterações ósseas e baixas concentrações séricas de vitamina D em pacientes com doença inflamatória intestinal

Introdução: A doença inflamatória intestinal (DII), como a doença de Crohn (DC) e a retocolite ulcerativa (RU), caracterizam-se pela inflamação crônica no intestino, que pode reduzir a absorção de vitamina D e cálcio.
Objetivo: Investigar as alterações ósseas presentes em pacientes com DII e as dosagens séricas de vitamina D.
Método: Estudo transversal analítico baseado na revisão de prontuários de pacientes com DII de um consultório privado de Curitiba, PR. Em todos os pacientes, foram dosadas as concentrações séricas de vitamina D e foi feita a densitometria óssea. Cento e cinco pacientes foram incluídos no estudo, dos quais 38 (58,4%) foram diagnosticados com DC, 27 (41.6%) com RU e 40 com síndrome do intestino irritável (SII) como grupo de comparação.
Resultados: Quando comparados com pacientes com RU, os pacientes com DC apresentaram maior prevalência de alterações ósseas, sendo 15,8% com osteoporose e 36,8% com osteopenia. Na RU, as alterações ósseas ocorreram em 29,6% dos casos, 3,7% com osteoporose e 25,9% com osteopenia. Em relação às dosagens de vitamina D, dentre os pacientes com DC, 10,5% apresentavam deficiência, 65,8%, insuficiência e 23,7%, suficiência. Na RU, 7,4% dos casos tinham deficiência, 74,1%, insuficiência e 18,5%, suficiência. No grupo com SII, observaram-se deficiência em 17,5%, insuficiência em 55% e suficiên-

cia em 27,5%. Não foi observada diferença significativa entre os grupos.
Conclusão: Pacientes com DII apresentaram alta prevalência de alterações ósseas, principalmente aqueles com DC. As concentrações séricas de vitamina D estão abaixo do recomendado em todos os grupos avaliados.

Palavras-chave: doença inflamatória intestinal, osteoporose, vitamina D.

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High cervical spine spondylodiscitis management and literature review

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Summary

Spondylodiscitis affecting the cervical spine is the most unusual type. Disease progression can be dramatic, even causing quadriplegia and death. We present an unusual case that progressed with osteolytic lesions between C2 and C3, causing cord compression and epidural abscess. The patient was treated surgically by a double approach and improved without neurological deficits and with better inflammatory markers. We reviewed the current literature on the subject.

Keywords: spinal disease, neck pain, discitis.

Introduction

Pyogenic spinal infections are rare and affect 1 to 7% of all cases of osteomyelitis. However, its incidence has been increasing, mainly due to the increased longevity of the population and a higher incidence of comorbidities that cause immunosuppression.[1] Discitis predominantly occurs in the lumbar spine, followed by the thoracic spine and, to a lesser extent, the cervical spine.[2] The literature regarding cervical spondylodiscitis is scarce. Its presentation may be more dramatic and with rapid evolution, causing early neurological deficits. Emergency treatment is mandatory, since it can progress to fulminant sepsis and neurological complications.[3]

We are reporting an unusual case of cervical pyogenic spondylodiscitis. The literature was revised in order to better understand the subject.

Case report

Male patient, 59 years old, farmer. The initial complaint was intense neck pain for 2 months with progressive worsening. This was associated with constitutional symptoms including weight loss of 10 kg in 45 days, loss of appetite, fever, adynamia, and night sweats. Upon physical examination, the patient was emaciated, febrile (38.3°C), prostrate, and presented intense pain upon anterior cervical palpation with an antalgic posture in semiflexion. Passive and active cervical mobilization was painful. Muscle strength, deep tendon reflexes, and sensitivity in the limbs were preserved.

Laboratory tests on admission showed 8,200 leukocytes with 2% Auer rods, erythrocyte sedimentation rate (ESR) of 100 mm/h and c-reactive protein (CRP) of 35 mg/L. Blood culture from two samples showed no growth of microorganisms.

X-ray and computerized axial tomography of the cervical spine showed lytic lesions between C2 and C3. The nuclear magnetic resonance imaging (NMR) showed signs suggestive of spondylodiscitis in C2-C3 associated with the presence of a massive epidural abscess compressing the ventral surface of the spinal cord, as well as involvement of paravertebral and prevertebral soft tissues (Figure 1). Bone scintigraphy ruled out an expansive tumor process and confirmed the NMR findings.

A decompressive anterior cervicotomy was performed at C2-C3 level, with the presence of purulent material. A discectomy was performed and a tricortical structural iliac graft was inserted. After 2 weeks, a posterior cervical arthrodesis was performed with C2-C3 sublaminar wiring associated with insertion of a posterior iliac tricortical graft block (Figures 2 and 3). Examinations showed the

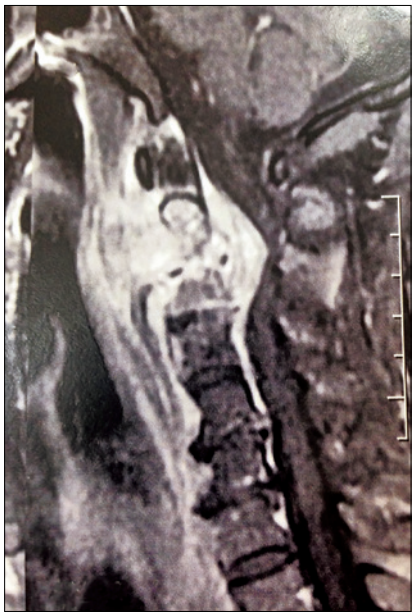

FIGURE 1 Cervical NMR image showing signs of spondylodiscitis in C2-C3 with spinal cord compression and epidural abscess.

growth of multisensitive *Staphylococcus aureus* and anatomopathological examination confirmed inflammation.

In accordance with the sensitivity spectrum of the germ, oxacillin 500 mg, intravenous, every 4 hours was given for 3 weeks, followed by cefalexin 500 mg, peroral, every 6 hours for another 5 weeks, with a total of 8 weeks of antibiotic therapy.

The patient progressed satisfactorily with improvement of the pain, as assessed using a visual pain scale, which was 10 in the preoperative period and fell to 2 on the 5th day after surgery. The Oswestry 2.0 questionnaires were also applied before and after surgery, with 49/50 points in the preoperative period, ranking as an invalid, and 4/50 in the postoperative period, showing an excellent post-surgical outcome. The patient was discharged after three weeks without complaints and with laboratory examinations that showed a decrease in inflammatory markers. Six weeks after surgery, the patient was still using a Philadelphia cervical collar, and already showed signs of osseointegration of the graft. The patient remained asymptomatic.

Discussion

Cervical spondylodiscitis is rare, given that most vertebral abscess and cases of discitis occur in the thoracic and lumbar spine. The annual incidence varies from 0.5 to 2.5 cases per 100,000 inhabitants. Spondylodiscitis is the primary manifestation of hematogenous osteomyelitis in patients over 50 years of age, representing 3 to 5% of all cases of osteomyelitis.[4]

Pathogens can affect the spine by three routes: hematogenous, external inoculation or contiguity.

The arterial hematogenous route is the predominant one, enabling the infection to be disseminated from distant sites.[5]

Although a broad spectrum of microorganisms have been identified (bacteria, mycobacteria, fungi and parasites), a monobacterial etiology predominates, with *S. aureus* being the most common.[6] The present case reiterates the higher prevalence of this germ in the literature.

The main risk factors include use of intravenous drugs and comorbidities such as diabetes and terminal chronic renal failure.[6] We found no risk factors in the case reported, which makes it even more atypical.

As shown in the literature,[5] NMR is the most sensitive (93-96%) and specific (92.5-97%) test for the early detection of spondylodiscitis. In most cases, it can differentiate between pyogenic infections, neoplasms, and tuberculosis.

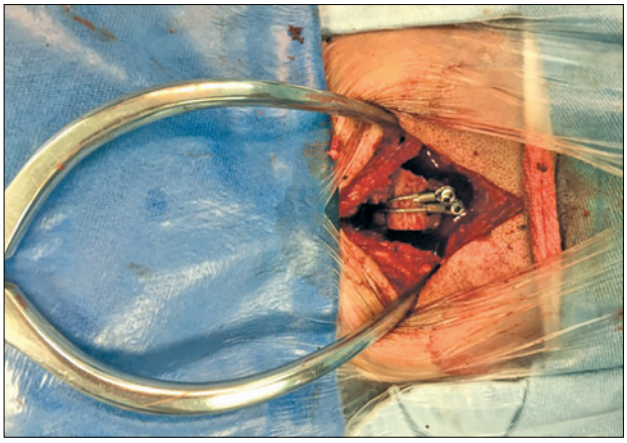

FIGURE 2 Transoperative image of posterior fixation with the iliac graft.

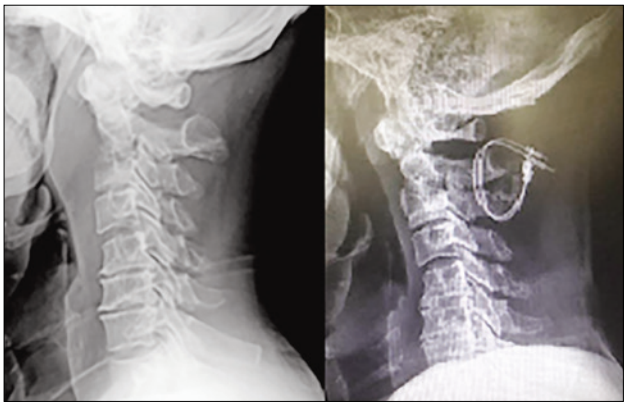

FIGURE 3 Profile image of the pre and postoperative radiography.

Furthermore, it can define the paravertebral and epidural spaces better.[7] This complementary examination was used to diagnose a case that progressed slowly and was fundamental for the diagnostic definition, treatment and resolution of the case.

In order to direct the antibiotic therapy, a percutaneous biopsy, which is a safe and minimally invasive procedure, may bey performed. If the first sample is negative, some experts recommend taking another.[8] Friedman reported 50% positivity in cultures produced from percutaneous biopsies. Surgical debridement is reserved for patients who present abscesses and neural compression and need to have their spine stabilized, a fact that occurred in our case. Other indications include debridement of devitalized tissue and removal of infected implant material. Specific antibiotics should be administered for 8 to 12 weeks after surgery, according to the results of the culture. Infections in most of the patients are resolved using this approach.[9]

Classically, the standard treatment was corpectomy and placement of structured grafts without the use of the implant material. Currently, most surgeons have preferred techniques that provide greater stability to the targeted site using implant material in patients treated for spondylodiscitis. There is preference for the posterior route in the cervical spine in order to avoid the main complications, which include graft migration, failure of the synthesis material and esophageal fistula. Several authors have reported no complications related to the use of these implants.[10]

Our patient was treated according to this protocol and recovered satisfactorily.

Conclusion

Although cervical spondylodiscitis is a rare disease, it is a diagnosis that should not be overlooked in patients who have indolent neck pain associated with constitutional symptoms. Early diagnosis and initiation of therapy are the only means of avoiding disease progression, thus preventing patients from having sequelae that are often irreversible.

Resumo

Espondilodiscite da coluna cervical alta: manejo e revisão da literatura

A espondilodiscite, que acomete a coluna cervical, é a de localização mais rara. Pode ter uma evolução dramática, inclusive causando tetraplegia e óbito. Apresentamos um caso atípico que evoluiu com lesões osteolíticas entre C2 e C3, causando compressão medular e abscesso epidural. O paciente foi submetido a tratamento cirúrgico por dupla abordagem e evoluiu bem, sem déficits neurológicos e com melhora dos marcadores inflamatórios. Revisamos a literatura vigente sobre o assunto.

Palavras-chave: doenças da coluna vertebral, cervicalgia, discite.

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Depression, stress and anxiety in medical students: A cross-sectional comparison between students from different semesters

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Summary

Objective: To compare the prevalence of anxiety, depression, and stress in medical students from all semesters of a Brazilian medical school and assess their respective associated factors.
Method: A cross-sectional study of students from the twelve semesters of a Brazilian medical school was carried out. Students filled out a questionnaire including sociodemographics, religiosity (DUREL – Duke Religion Index), and mental health (DASS-21 – Depression, Anxiety, and Stress Scale). The students were compared for mental health variables (Chi-squared/ANOVA). Linear regression models were employed to assess factors associated with DASS-21 scores.
Results: 761 (75.4%) students answered the questionnaire; 34.6% reported depressive symptomatology, 37.2% showed anxiety symptoms, and 47.1% stress symptoms. Significant differences were found for: anxiety – ANOVA: [F = 2.536, p=0.004] between first and tenth (p=0.048) and first and eleventh (p=0.025) semesters; depression – ANOVA: [F = 2.410, p=0.006] between first and second semesters (p=0.045); and stress – ANOVA: [F = 2.968, p=0.001] between seventh and twelfth (p=0.044), tenth and twelfth (p=0.011), and eleventh and twelfth (p=0.001) semesters. The following factors were associated with (a) stress: female gender, anxiety, and depression; (b) depression: female gender, intrinsic religiosity, anxiety, and stress; and (c) anxiety: course semester, depression, and stress.
Conclusion: Our findings revealed high levels of depression, anxiety, and stress symptoms in medical students, with marked differences among course semesters. Gender and religiosity appeared to influence the mental health of the medical students.

Keywords: medical education, depression, anxiety, stress, medical students.

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Introduction

University life during medical training entails full-time commitment and responsibility of undergraduates regarding academic tasks and care provided to patients and their companions. Long working and study hours, environments not ideally suited to learning, sleep deprivation, as well as factors interfering in everyday personal life are common during this period.[1] These aspects, associated with a lack of factors promoting quality of life, can lead to stress levels that negatively impact the physical, mental, and emotional health of students, compromising their academic performance.[2]

Emotional disorders in medical undergraduates are not uncommon, influencing mental health as well as honesty, academic performance, and use of alcohol and other drugs.[3] In Europe, around 30% of medical students suffer from depression or anxiety,[4] a rate similar to that reported by Brazilian studies, in which 20 to 50% of medical students were found to present with mood disorders.[5,6] Medical students also have higher rates of depression and suicidal ideation than the general population, posing a major challenge to the training of future physicians.[2]

Further studies elucidating the factors associated with mental disorders that affect performance and quality of life of undergraduate medical students are needed. Within this context, stress plays a major role in this interrelationship, most likely stemming from the frequent

exposure to pain, suffering and death.[7] The high levels of stress in medical students are important predictors of anxiety and depression.[8,9]

In fact, health, work and quality of life are intrinsically linked, where higher levels of stress lead to a decline in quality of life due to demotivation, irritation, depression, and unhappy personal life, impacting interaction of the individuals at different points in their life.[10] Thus, stress impairs learning, since this directly interferes in the brain's executive functions and can affect the performance of undergraduate students at specific times or throughout their academic course.[11]

In this respect, there appears to be a relationship between the course year and severity of mental disorders.[12] In a four-year cohort study, mild-to-moderate depression rose from 4 to 12% during medical school training. Similarly, another study[13] reported an increase in mild depression from 4.3 to 11.2%, and in moderate depression from 1.7 to 6.9%, by the end of the first year of medical school.

Ascertaining the times during which students are most susceptible to psychiatric disturbances may help in the implementation of strategies to promote physical, mental, emotional, and spiritual well-being. These strategies might include both individuals and the group as a whole,[14,15] promoting the resumption of healthy habits, and attention to health, leisure, and religiosity.[16-18]

The objective of the present study was to compare the prevalence of anxiety, depression, and stress in medical students from all semesters of medical school training and assess their respective associated factors to evaluate potential stressors and identify the major challenges faced by the students during medical training. Ultimately, the results can aid in the planning and development of more effective intervention and prevention programs and in the implementation of more balanced medical curricula.[19]

Method

Study design and participants

This cross-sectional study included students from the six years of the medical course of the Federal University of Juiz de Fora (Brazil) and was conducted between September and November 2014. The medical course spans six years (comprising twelve separate semesters) typically divided into three stages, each averaging two years: pre-clinical, clinical, and internship.

All students officially enrolled in the medical course were invited to take part. Students away from the city of Juiz de Fora on governmental international exchange programs Sciences Without Borders or undertaking optional internships, individuals not present at the time of data collection, and those who did not complete the questionnaire or refused to take part in the study were excluded.

The project was approved by the Research Ethics Committee of the University Teaching Hospital/UFJF under report n° 790.822 and all participating students signed the informed consent form.

Instruments

The self-report questionnaire employed took approximately 20 minutes to answer and collected data on:

- Sociodemographics: age, ethnicity/race, marital status, employment status, family income.
- Religiosity: the Duke Religion Index was used, a five-item measure of three different dimensions of religious involvement: one question (with six possible answers) for organizational religiousness (OR)—religious attendance; one question (with six possible answers) for non-organizational religiousness (NOR)— religious activities performed in private, such as prayer, Scripture study, watching religious TV or listening to religious radio, and three questions (with five possible answers each) for intrinsic religiousness (IR)—pursuing religion as an ultimate end in itself. For the intrinsic religiosity score, we summed the three questions (possible score 3 to 15), in which higher scores indicate higher levels of religiosity. This scale has been previously validated for use in Brazil.[20]
- Depression, anxiety, and stress: assessed by the DASS 21 – Depression, Anxiety and Stress Scale validated for Portuguese.[21] This 21-item short scale allows simultaneous assessment of the three emotional states of depression, anxiety, and stress, is easy to apply in both clinical and non-clinical settings, and suitable for use in different age groups,[21] including medical students.

Procedures

Data collection took place during class time (before or after educational activities) and the questionnaire was applied as follows: the researchers explained the objectives of the study, asked students to fill out the questionnaire and sign the consent form, and guaranteed confidentiality of the data. The questionnaires were applied mid-way through the academic semester in order to assess students at a time nearest their basal state, i.e. outside exam time (end of semester) and resumption of classes (beginning of semester).

Statistical analysis

The data collected were keyed into Excel for Windows and statistical analysis was carried out using the software package SPSS version 17.0 (SPSS Inc.). Descriptive analysis with measurements of frequency, mean, and standard deviation was used to express sociodemographic variables and results from the scales and questionnaires.

The students from the 12 semesters of the course were compared for demographic, socioeconomic, and mental health variables using the Chi-squared or ANOVA tests. When significant difference was detected by ANOVA, a post-hoc test (Bonferroni) was performed.

Linear regression models were employed to assess the factors associated with each dependent variable (stress, depression, and anxiety), including goodness-of-fit measures and assessment of R^2. The following variables were entered in each step:

- Model 1: gender, semester, age, family income, organizational religiosity, non-organizational religiosity, and intrinsic religiosity.
- Model 2: DASS Depression, DASS anxiety or DASS Stress according to the dependent variable.

A value of $p<0.05$ and confidence interval of 95% were adopted for all analyses.

Results

Of the total 1,009 students enrolled at the medical course, 75.4% (n=761) answered the questionnaire, comprising 298 (39.1%) at pre-clinical, 244 (32%) at clinical, and 219 (28,7%) at internship stages.

The profile of the sample studied was predominantly women (55.8%) with a mean age of 22.1 (SD=3.3) years, white (75.7%), and with a family income of > eight Brazilian minimum wages (> US$ 2,000.00) (50.7%). With regard to religious aspects, 51.2% of the students reported being Catholics, 17.1% no religion, 11% Evangelical or Protestant, 8.3% Spiritists, and 6.4% stated they did not believe in God. Sociodemographic data for the sample are given in Table 1.

With regard to the prevalence of depression, anxiety, and stress, as assessed by the DASS-21, 34.6% had depressive symptomatology (8.8% severe or extremely severe), 37.2% had anxiety symptoms (12.2% severe or extremely severe), and 47.1% had stress symptoms (17.4% severe or extremely severe). The constructs anxiety, depression, and stress were highly correlated, with values ranging from r=0.554 to 0.696 ($p<0.01$).

TABLE 1 Sociodemographic and religious characteristics of medical undergraduate students.

Variable	
Gender (n, %)	
Female	425 (55.8%)
Male	336 (44.2%)
Age (Mean, SD)	22.10 (3.34)
Stage of course (n, %)	
Pre-clinical (1^{st}-4^{th})	298 (39.1%)
Clinical (5^{th}-8^{th})	244 (32%)
Internship (9^{th}-12^{th})	219 (28.7%)
Ethnicity/Race (n, %)	
White	576 (75.7%)
Black	32 (4.2%)
Mulatto	61 (8%)
Others	92 (12.1%)
Family income (n, %)	
Up to 3 minimum wages	105 (13.8%)
4-7 minimum wages	261 (34.3%)
8-12 minimum wages	207 (27.2%)
> 12 minimum wages	179 (23.5%)
Not reported	1 (0.1%)
Religious affiliation (n, %)	
None, but believe in God	130 (17.1%)
None, and do not believe in God	49 (6.4%)
Roman Catholic	390 (51.2%)
Evangelical/Protestant	84 (11.1%)
Spiritist	63 (8.3%)
Others	27 (3.5%)
Not reported	18 (2.4%)

Figure 1 (A to C) shows the differences in mean depression, anxiety, and stress for the different semesters. Significant differences in means were detected for:

a. anxiety – ANOVA: $F(11,743) = 2.536$, $p=0.004$, between first and tenth ($p=0.048$), and first and eleventh ($p=0.025$) semesters;
b. depression – ANOVA: $F(11,745) = 2.410$, $p=0.006$, between first and second semesters ($p=0.045$);
c. stress – ANOVA: $F(11,744) = 2.968$, $p=0.001$, between seventh and twelfth ($p=0.044$), tenth and twelfth ($p=0.011$), and eleventh and twelfth ($p=0.001$) semesters.

Linear regression (Table 2) revealed associations between DASS-21 scores for:

- DASS Stress: with female gender (B=0.87, SE: 0.24, $p<0.001$), DASS Anxiety (B=0.63, SE: 0.03, $p<0.001$) and DASS Depression (B=0.40, SE: 0.03, $p<0.001$) – $R^2=0.568$;

TABLE 2 Linear regression analysis of association of DASS scores with sociodemographic, religiousness, and mental health among medical undergraduate students.

	Model 1			Model 2		
Stress[a]	**B**	**SE**	**p**	**B**	**SE**	**p**
Gender (female)	1.46	0.37	**<0.001**	0.87	0.24	**<0.001**
Semester	-0.04	0.06	0.472	0.05	0.04	0.220
Age	0.005	0.06	0.941	-0.02	0.04	0.543
Family income	-0.05	0.17	0.770	0.08	0.11	0.456
Religious attendance	0.15	0.18	0.394	0.03	0.12	0.775
Non-organizational religiousness	-0.18	0.14	0.186	-0.07	0.09	0.411
Intrinsic religiousness	0.03	0.08	0.677	-0.06	0.05	0.253
DASS Anxiety				0.63	0.03	**<0.001**
DASS Depression				0.40	0.03	**<0.001**
Depression[b]	**B**	**SE**	**p**	**B**	**SE**	**p**
Gender (female)	0.24	0.32	0.459	0.56	0.24	**0.023**
Semester	-0.44	0.05	0.412	0.07	0.04	0.870
Age	0.74	0.05	0.190	0.07	0.04	0.094
Family income	-0.16	0.15	0.281	-0.11	0.11	0.328
Religious attendance	0.08	0.16	0.599	-0.01	0.122	0.891
Non-organizational religiousness	-0.16	0.12	0.191	-0.06	0.09	0.476
Intrinsic religiousness	-0.23	0.07	**0.001**	-0.22	0.05	**<0.001**
DASS Stress				0.40	0.03	**<0.001**
DASS Anxiety				0.27	0.04	**<0.001**
Anxiety[c]	**B**	**SE**	**p**	**B**	**SE**	**p**
Gender (female)	0.78	0.28	**0.007**	0.10	0.20	0.611
Semester	-0.12	0.04	**0.011**	-0.94	0.03	**<0.001**
Age	0.001	0.05	0.978	-0.01	0.03	0.684
Family income	-0.11	0.13	0.407	-0.05	0.09	0.535
Religious attendance	0.13	0.14	0.331	0.05	0.10	0.584
Non-organizational religiousness	-0.07	0.11	0.524	0.04	0.07	0.598
Intrinsic religiousness	0.000	0.06	1.000	-0.05	0.04	0.193
DASS Stress				0.18	0.03	**<0.001**
DASS Depression				0.43	0.02	**<0.001**

[a]Model 1: R square=0.030; Model 2: R square=0.516.
[b]Model 1: R square=0.036; Model 2: R square=0.446.
[c]Model 1: R square=0.020; Model 2: R square=0.568.

- DASS Depression: with female gender (B=0.56, SE: 0.24, p=0.023), intrinsic religiosity (B=0.22, SE: 0.05, p<0.001), DASS Anxiety (B=0.27, SE: 0.04, p<0.001), and DASS Stress (B=0.40, SE: 0.03, p<0.001) – R^2=0.446;
- DASS Anxiety: with semester of course (B=-0.94, SE: 0.03, p<0.001), DASS Depression (B=0.43, SE: 0.02, p<0.001) and DASS Stress (B=0.18, SE: 0.03, p<0.001) – R^2=0.516.

Discussion

The results of this study revealed a high prevalence of anxiety, depression, and stress among medical students, with statistically significant differences for course semesters, and the influence of several factors such as gender and religiosity on the students' emotional state. Medical schools are known to be environments associated with stressors and factors that negatively impact academic performance as well as both physical and emotional health of students.[9,22,23]

With regard to the changes in mental health found for the different semesters, students from the first semester commenced activities with a high level of anxiety, exceeding that of students from the tenth and eleventh semesters, most likely stemming from the experience of

the unknown, from the expectations of embarking on this new path, and from moving away from home (as it happens to most students in our university). Miller et al.,[24] investigating first-year students, postulated that stress in this group stemmed from the nature and overload of the work inherent to the medical course, as well as from the academic structure of the course and its teaching methods. In fact, embarking on a medical course is fraught with numerous challenges beginning with the selection process, undeniably competitive and with a high cut-off point, particularly at Brazilian public institutions. On top of this, there is a certain glamour conferred by society to undergraduate medical students grounded in the dream of implicit economic success, which could lead to an over-expectation and frustration.[25] This phenomenon has been corroborated by various studies.[6,26] A study conducted in Brazil found a higher prevalence of anxiety symptoms in first-year (30.8%) medical students compared to sixth-year (9.4%) medical students,[6] while an investigation in Malaysia showed that anxious symptomatology was much more prevalent than depressive symptoms, and that stress in students newly enrolling in medical school was greater than students at the latter stages of the course.[27]

Regarding depressive symptoms, it is noteworthy that students in the present study exhibited a sharp rise in the second period of the course compared with the first. At the outset of the first year, we believe two contradictory feelings come to the fore in the students: on the one hand there is the euphoria of approval, reinforcing the dream of the chosen profession, while on the other there is the frustration of commencing a course with an eminently theoretical content and few practical activities, closely resembling the recently experienced middle school education. The introjected and oft replicated discourse that being a physician demands the abdication of social and personal life, and multiple sacrifices can exacerbate this initial discomfort.[28] Similar data was found by Wolf et al.[29] with reports that first-year students complained of the scant opportunities to pursue their personal relationships. The authors also revealed that, at the end of the first year, students presented more depressive symptoms, which peaked at the end of the second year in the fourth semester. Similarly, Quince et al.[30] performed a longitudinal study at a UK medical school and found a prevalence of depression ranging from 5.7 to 10.6% in students on the basic years and 2.7 to 8.2% in students on the clinical stages of the course.

In the present sample, the high levels of stress across all semesters of the course are illustrated in Figure 1, with a notable rise during the eighth semester of the fourth year, marking the end of the pre-clinical stage (entry to the clinical or internship/clerkship stage), and during the twelfth period prior to graduation. A substantial decline in stress can also be noted in the tenth and eleventh semesters. During the twelfth semester, the final semester before graduation, the students normally prepare for course conclusion and assume the commitment of practicing the profession. This period is marked by the phase of preparing for the medical residency tests in the chosen area of specialization, where the selection processes are highly competitive and the residency positions are in short supply. The increased distress and lack of time, coupled with the conflicts of choosing a specialty, explain the predominance of stress in internship candidates, a situation confirmed by other authors.[31] A study in three British universities involving fourth-year medical students found a 31.2% prevalence of emotional disturbances, a similar rate to those found in American studies.[8] Stress was also found to correlate with depression and anxiety, showing the inter-dependence of these symptoms.

With regard to the factors associated with the mental health of these students, there was an association of gender with both depression and stress. Studies show that women have a greater prevalence of depression and anxiety in the general population, although data for medical students are conflicting. Notwithstanding, a systematic review[3] revealed that half of the studies published involving medical students reported difference in depression and stress between genders, corroborated by the present findings.

Another associated factor was intrinsic religiosity. Although many studies point to an association of religious/spiritual beliefs with mental health, physical health, and quality of life[32,33] and other investigations have assessed religiosity and medical students' views on this issue,[34] few studies have specifically investigated the association between mental health and beliefs in medical education. Wachholtz et al.[35] assessed 259 American medical students and found that individuals with lower levels of spiritual well-being and daily spiritual experiences had greater psychological stress and burnout. Similarly, Vasegh et al.[36] found a negative correlation among religiosity, depression, and anxiety in 285 Iranian students, which is in accordance to our findings. By contrast, Lupo et al.[37] conducted a study of 119 medical students in Israel and found no association between religiosity, depression, and anxiety showing these results can be reduced or augmented according to culture and religious affiliation.

The present study has several limitations. This was a cross-sectional study, thereby precluding conclusions on causality and weakening the comparison among semesters, since these involved different student groups. However,

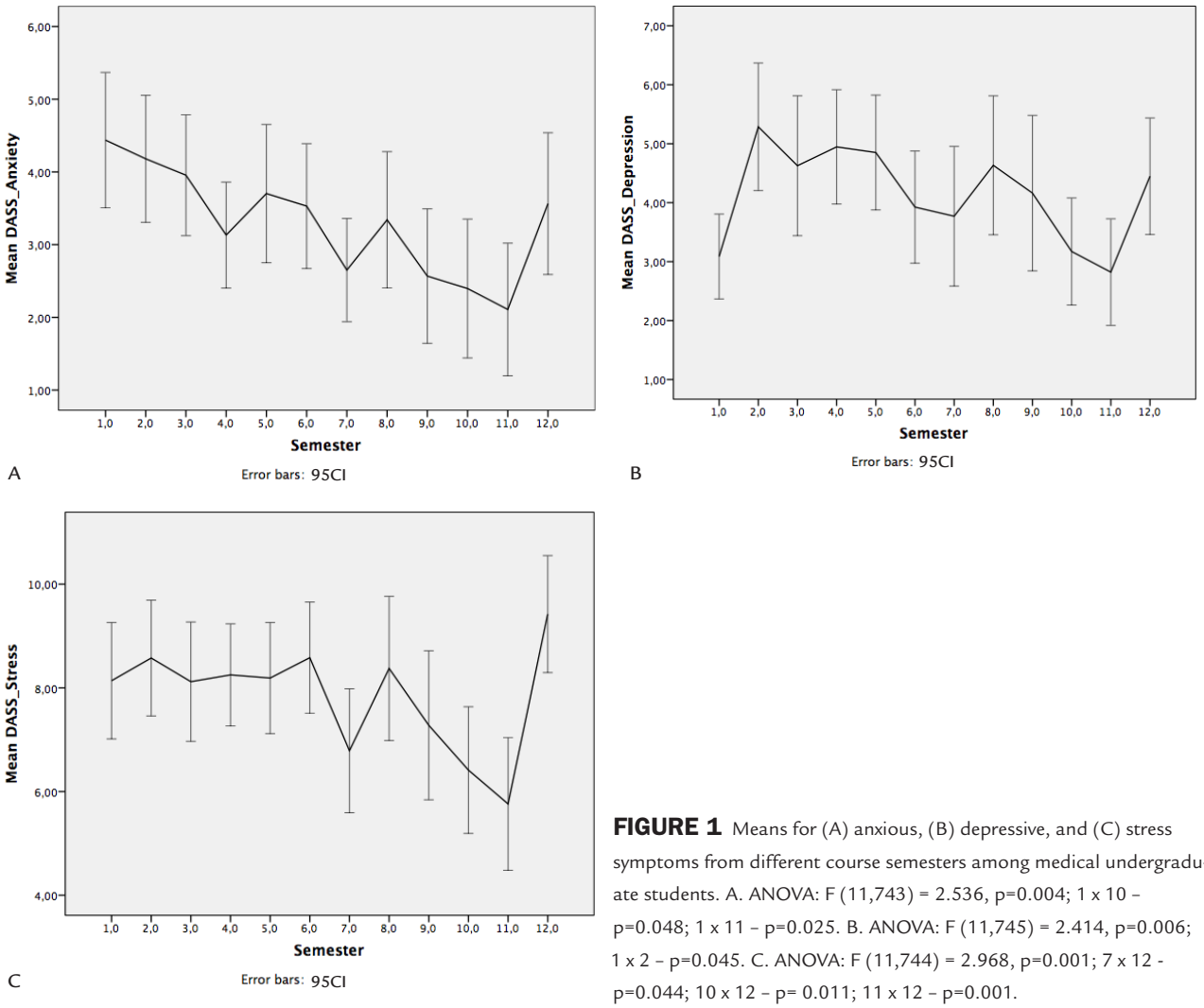

FIGURE 1 Means for (A) anxious, (B) depressive, and (C) stress symptoms from different course semesters among medical undergraduate students. A. ANOVA: F (11,743) = 2.536, p=0.004; 1 x 10 – p=0.048; 1 x 11 – p=0.025. B. ANOVA: F (11,745) = 2.414, p=0.006; 1 x 2 – p=0.045. C. ANOVA: F (11,744) = 2.968, p=0.001; 7 x 12 - p=0.044; 10 x 12 – p= 0.011; 11 x 12 – p=0.001.

this type of methodology has been previously used in many other studies on medical education.[6] Nevertheless, despite the high response rate of over 70%, some students with mental health-related problems may have refused to take part in the study. Since the present study was carried out in one Brazilian medical school, generalization should be made with caution.

On the other hand, the study also has noteworthy strengths. The sample size was suitable for assessments among semesters, while the validated scales employed are used worldwide, easily applicable, and can be reproduced by other schools of medicine. Further follow-up studies are needed to elucidate the course of anxiety, depression, and stress in this population. Future investigations could provide a better understanding of the factors influencing the mental health of these students and aid the planning of interventions to help them cope with the challenges faced.

The present study's findings revealed high levels of depressive, anxiety, and stress symptoms in medical students, with marked differences for course semesters. Factors such as gender and religiosity appeared to influence the mental health of the medical students. These results may help in devising preventive strategies and early identification of students at risk for mental health decline during the course of medical training.

Conflict of interest

The authors declare no conflict of interest.

Resumo

Objetivo: Comparar a prevalência de ansiedade, depressão e estresse em estudantes de todos os períodos de graduação de medicina e avaliar os fatores associados.

Método: Estudo transversal e comparativo, realizado com alunos dos seis anos (doze semestres) de um curso de medicina brasileiro. Os alunos preencheram um questionário com dados sociodemográficos e relacionados à religiosidade (Duke Religion Index) e à saúde mental (DASS-21 – Depression, Anxiety and Stress Scale). Os estudantes dos 12 períodos do curso foram comparados quanto às variáveis demográficas, socioeconômicas e saúde mental por meio dos testes de Qui-quadrado ou ANOVA. Para avaliar os fatores associados a cada variável dependente (estresse, depressão e ansiedade), foram utilizados modelos de regressão linear.

Resultados: Responderam ao questionário 743 (73,63%) dos 1.009 estudantes matriculados no curso de medicina, com 34,6% apresentando sintomatologia depressiva; 37,2%, sintomas de ansiedade; e 47,1%, estresse. Houve diferenças significantes entre os períodos em relação à ansiedade – ANOVA: (F = 2,536; p=0,004), sendo as diferenças entre o primeiro e o décimo período (p=0,048) e entre o primeiro e o décimo primeiro período (p=0,025); à depressão – ANOVA: (F = 2,410; p=0,006), sendo as diferenças entre o primeiro e o segundo período (p=0,045); e ao estresse – ANOVA: (F = 2,968; p=0,001), sendo as diferenças entre o sétimo e o décimo segundo período (p=0,044), entre o décimo e o décimo segundo (p=0,011) e entre o décimo primeiro e o décimo segundo (p=0,001). Estiveram associados (a) ao estresse: gênero feminino, ansiedade e depressão; (b) à depressão: gênero feminino, religiosidade intrínseca, ansiedade e estresse; (c) à ansiedade: semestre do curso, depressão e estresse.

Conclusão: Os achados do presente estudo mostram altos níveis de sintomas de depressão, ansiedade e estresse em estudantes de medicina, com diferenças marcantes nos diferentes semestres do curso. Fatores como gênero e religiosidade parecem influenciar a saúde mental dos estudantes de medicina.

Palavras-chave: educação médica, depressão, ansiedade, estresse, estudantes de medicina.

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Experimental burns: Comparison between silver sulfadiazine and photobiomodulation

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Summary

Objective: To analyze morphological characteristics and organization of the collagen fibers of third degree burns from scalding compared to laser therapy and silver sulfadiazine, the latter considered as the gold standard.
Method: Were selected 12 animals (*Rattus norvegicus*) also divided into three groups (control group [CG] – untreated burns; sulfadiazine group [SG] – burns were treated with silver sulfadiazine at 1%; laser group [LG] – burns were treated with photobiomodulation). The scald burns were carried out by using PVC mold, and the material collected on the 14th day after burn was prepared for morphological and optical retardation analysis for evaluation of inflammatory infiltrates and collagen organization, respectively.
Results: On the 14th day, the laser and sulfadiazine groups had mild inflammatory response, while the control group showed an intense inflammatory process, with statistical significance between laser and control groups, but not between sulfadiazine and control groups. Laser and sulfadiazine groups no longer had granulation tissue, opposite to what was seen in the control group. The presence of hair follicles and ulcer did not significantly differ between groups. The optical retardation of collagen fibers was higher in sulfadiazine group, followed by laser and control groups. As for systemic effect, we were able to identify it by simply analyzing the presence or absence of granulation tissue.
Conclusion: Morphologically, the laser or silver sulfadiazine treatments were similar and both provided better organization of collagen fibers in relation to the untreated group. However, the sulfadiazine group modulated the deposition of collagen fibers more efficiently than the laser group.

Keywords: burns, scald, sulfadiazine, laser, collagen, rats.

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Introduction

Burns are a major social and public health problem, given that any person may be subjected to such injury, regardless of age, sex, color or social class.[1] Burn accidents affect 11 million people worldwide, and these figures refer only to those who have suffered burns severe enough to seek medical attention.[2] In this type of accident, there is the question of physical and psychological sequelae that may persist in the victim, such as the change of their own image, in addition to social and economic issues, given that it involves the injured patient's reintegration into society, and the high cost of keeping them in hospitals.[3,4] Burns may be caused by thermal, chemical, electrical or radioactive agents that act on the tissue covering the body, causing lesions that partially or fully affect the skin, and which can reach more internal tissues, such as subcutaneous tissue, muscles, tendons, and bones.[5] Thermal burns are the most common, with flames and overheated substances as the main agents.[6] This type of lesion may present in different levels of severity and with a range of complications, making treatment difficult. They can be classified into first degree, second degree and third degree according to their depth of involvement.[7] The healing of burned skin occurs in the same way as any other injury, following the same steps of repair

– inflammation, proliferation, and remodeling – and an immature scar may exhibit signs and symptoms that include erythema, progressive elevation in the form of a tumor, edema, pruritus, pain, and blistering. As it matures, the lesion becomes asymptomatic, more malleable, presenting a color close to that of the patient's skin, with better tensile strength.[8]

A recent study involved a review of the literature on treatments offered for dealing with burns in the acute phase, and the topical agents used for the most commonly found dressings were associations of neomycin sulfate and bacitracin, clostebol acetate and neomycin sulfate, and silver sulfadiazine, which is still widely used in Brazil.[9]

Third-degree burns have been a major focus of research and investigation, searching for new treatment methods in order to improve the care of burn patients and also provide greater speed for a satisfactory result without major functional and aesthetic sequelae.[10] Experimental research conducted on animals have shown that photobiomodulation modulates cellular activity, angiogenesis, synthesis, and collagen deposition, leading to faster closure of the burn and esthetically improved scarring compared to those not treated with photons.[11-13] The study of the influence of photobiomodulation on the cicatricial process in a scald model using animals will contribute to the search for a treatment that minimizes the physical and psychological sequelae that arise in burn victims. Furthermore, the spending on such victims is expected to decrease, lowering the length of hospitalization and, consequently, reintegrating them more quickly into society.

This is a translational study aimed at comparing burn treatments in an animal model, using the conventional method with 1% silver sulfadiazine and the alternative method with photobiomodulation. The objective was to analyze the morphological characteristics and organization of the collagen fibers of third degree burns caused by scalding, in relation to laser therapy and to that considered as the gold standard treatment, with the main motivation being the search for a more effective, quicker, and less painful treatment for burn victims.

Method

Twelve female *Rattus novergicus* Wistar rats with a body mass between 250 and 300 g kept in a vivarium at Universidade Nove de Julho (Uninove, São Paulo) were selected. The animals were kept in plastic cages, under ideal hygiene, lighting, and temperature conditions, with standard feed from the vivarium and water *ad libitum*. The animals were divided randomly and homogeneously into three groups: CG – control group: the burn was not treated; SG – sulfadiazine group: the burn was subjected to treatment with 1% silver sulfadiazine; and LG – laser group: the burn was submitted to photobiomodulation.

The burn procedure was performed under general anesthesia with intraperitoneal injection of a solution of ketamine (Dopalen Vetbrands, Jacareí, SP) at a dose of 80 mg/kg and xylazine (Anasedan Vetbrands, Jacareí, SP) at a dose of 10 mg/kg. After the anesthesia was applied, the animals' backs were trichotomized for realization of the thermal injury via scalding, where four circular regions of the back, with a radius of 0.5 cm and an area of 0.8 cm^2, remained in contact with hot water at 60°C for 45 seconds. These areas were delimited by inserting the animal into a mold made of PVC pipe with the same four areas of 0.8 cm^2 each, according to the methodology adapted from Cribbs et al.[14] (Figure 1 A and B).

Thus, each animal had four burns on the back, three of which were treated in the same way according to the experimental group. One of the burns, randomly chosen and standardized between the groups, was kept as the control of that specific animal for verification of the systemic effect of the treatments applied.

After creating the injuries, 1% silver sulfadiazine (ointment) was applied to SG in a uniform manner and covering the full extent of the lesion. For treatment in the LG, a red diode laser, $\lambda = 660 \pm 2$ nm, with an output power equal to 5 mW and a beam area of 0.04 cm^2 (Twin Laser, MMOptics, São Carlos, Brazil), was coupled to an optical system in order to obtain an expanded beam 0.8 cm^2.

The wound was therefore radiated in a uniform manner (Figure 1 C). The irradiations occurred on days 0, 5 and 12 post-injury, with radiant exposure of 1 J/cm^2 and irradiance of 6.25 mW/cm^2, with 160 seconds of exposure per lesion.

For the morphological analysis, the collected material was stained with hematoxylin and eosin, and the following characteristics were evaluated under transmitted light microscopy with 400x magnification (Carl Zeiss Pol-Interferencial Photomicroscope, Germany): a) inflammatory response, characterized by the presence of polymorphonuclear leukocytes, where a categorization was assigned to each of the following conditions: absent = 0, mild presence = 1, moderate presence = 2 or intense presence = 3; b) granulation tissue, characterized by the presence of fibroblasts and neovascularization, and categorized as 0 for absent and 1 for present; c) presence or absence of hair follicles; d) presence or absence of ulcers.

For analysis of the collagen organization, quantification of birefringence was performed, in nanometers, un-

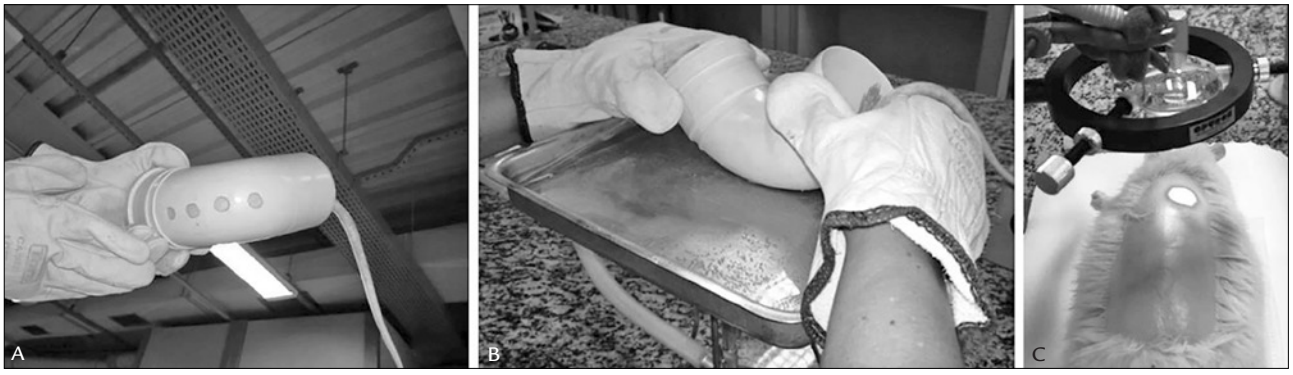

FIGURE 1 A. Mold for the scald. B. Induction of the scald with water at 60°C. C. Irradiation of lesions.

der a polarized and transmitted light microscope, with magnification of 400x, using an interference filter to obtain monochromatic light with l = 546 nm and compensator that introduces an optical retardation of l/4 (Carl Zeiss, Pol-Interferencial Photomicroscope, Germany).

The statistical evaluation of the morphology was performed to compare the groups on the 14th day post-burn, using the Chi-square test corrected by Fisher's exact test, with a significance level of 95%. This test is suitable for comparing the frequency of occurrence of an event which, in our case, the frequency of morphological categorizations.

For evaluation of birefringence, the Kruskal-Wallis H test for nonparametric data was used for the analysis between the groups, followed by the Mann-Whitney U test with a significance level of 95%. All of the analyses were performed using the software Minitab 16® (Minitab Inc., USA). All of the analyses were with the data from the 14th day post-burn.

Results

Table 1 shows the morphological result on day 14 post-induction of the burns.

According to Table 1, CG presented more polymorphonuclear leukocytes, and was categorized as having intense inflammatory activity, unlike LG, which had mild inflammation (p=0.002). However, there was no difference between CG and SG, since SG was characterized by intense (33% of the samples analyzed) and mild inflammation (67% of samples analyzed) (p=0.059), or between LG and SG (p=0.450). The control lesions of each animal did not respond differently compared to those of the full control group, showing an absence of a systemic effect when leukocytes were evaluated (p=0.468).

CG presented granulation tissue, unlike LG and SG (p=0.002). There was a statistically significant difference in relation to granulation tissue on the control injuries of each animal (absent) in relation to those of the full control group (present), indicating a systemic effect (p=0.009).

There were no differences regarding the presence of hair follicles between any of the groups (p=0.061). The three groups did not present ulcers on this day (p=0.182). There was no evidence of a systemic effect in these aspects (p=0.055).

Figure 2 presents the median values of the categorizations for each variable analyzed according to the groups, the analysis of the optical retardation of the collagen fibers, as well as photomicrographs representing each group.

The optical retardation of collagen fibers was greater in SG (24.24±6.82), followed by LG (30.30±6.82) and CG (42.42±10.60) (p=0.000). There was a significant difference between CG and LG (p=0.032), as well as between CG and SG (p=0.001). LG and SG also differed significantly (p=0.004).

Discussion

Our results indicate that on the 14th day the laser group already presented a mild inflammatory response, as well as the 1% silver sulfadiazine group (p=0.450), while the control group showed an intense inflammatory process, with statistical significance between LG and CG (p=0.002), but not between SG and CG (p=0.059). While LG and SG no longer presented granulation tissue on the 14th day, CG was still characterized by the presence of such. The presence of hair follicles did not differ significantly between the groups, although CG did not present follicles and the other groups had 67% of samples with hair follicles. In addition, significant differences were not detected in relation to the absence of ulcers between the groups.

The optical retardation of collagen fibers was greater in SG, followed by LG and CG (p=0.000).

As for systemic effect, this could only be identified by analyzing the presence or absence of granulation tissue, as the control burn of each animal in LG and SG pre-

TABLE 1 Morphological analysis on day 14 post-burn.

Groups	Inflammation	Granulation tissue	Hair follicle	Ulcer
	Categorization (percentage frequency)			
CG	Intense (100%)	Present (100%)	Absent (100%)	Absent (50%)
LG	Mild (100%)	Absent (100%)	Present (67%)	Absent (100%)
SG	Mild (67%) Intense (33%)	Absent (100%)	Present (67%)	Absent (100%)

CG: untreated control group; LG: photobiomodulation group; SG: silver sulfadiazine group.

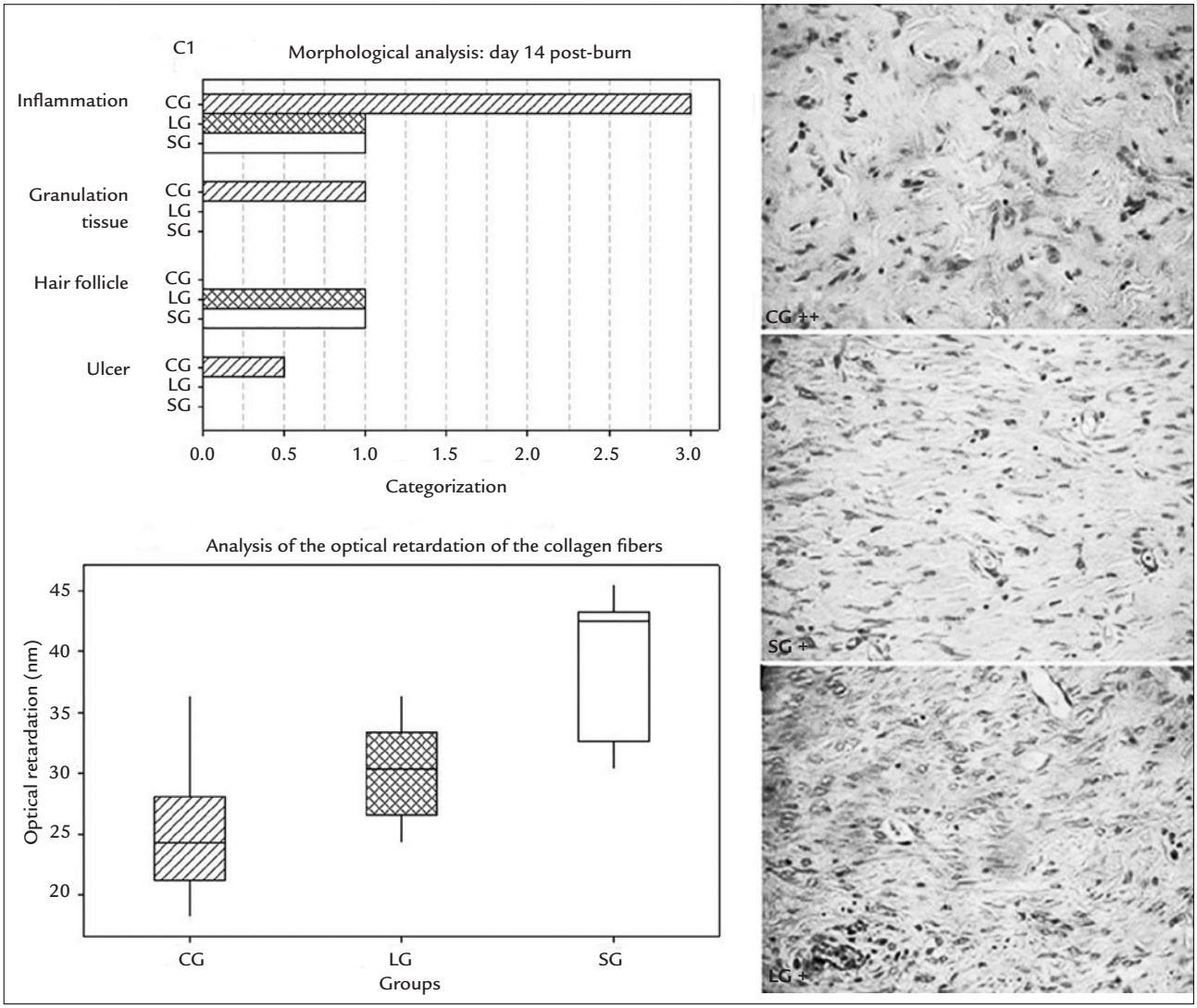

FIGURE 2 Median values of the categorizations attributed to inflammation, granulation tissue, hair follicles and ulceration. In the inferior graph, the horizontal lines from bottom to top represent the 25%, 50% and 75% of the optical retardation values measured in the samples. The photomicrographs to the right show the control group (CG), with an intense inflammatory process, the silver sulfadiazine group (SG) and the photobiomodulation group (LG) with mild processes. Staining with HE. 400x magnification.

sented no granulation tissue, while the burns in animals from the full CG still showed the stated tissue (p=0.009).

In the present study, the 14th day post-burn was chosen for analysis due to the occurrence of collagen deposition, given that after a third-degree burn collagen deposition is expected to be more evident on this day.[8] In this healing period, ulcers and granulation tissue are ideally absent (they should only be present on the first days of repair) and hair follicles begin to be perceived, with the inflammatory process already concluded or presented in a subtle manner.[15] Considering these facts, treatment with 1% silver sulfadiazine and photobiomodulation were shown to be efficient, especially for the laser group, which was the only to present mild inflammation on the day analyzed.

The correlation between optical retardation and collagen arrangement has been explored since the 1960s,[16,17] and, to this day, polarizing microscopy is an efficient method for quantifying the change in the birefringence of collagen due to the influence of different agents.[18] It is important to remember that better organization of collagen fibers implies a better appearance of the scar, as a consequence of a better cicatricial process.[8,19]

With respect to the systemic effect, there is no record of its occurrence in skin lesions using photobiomodulation. However, in this study, it could be observed in the group that was irradiated. A systemic effect of photobiomodulation has been observed when applied to bone tissue, according to the work of Coelho et al.[20]

Another important point to be considered is the dosimetry delivered to the tissue, given that the irradiation parameters were taken from a study that used cold burn instead of scalding.[13]

In burns caused by scalding there is dissipation of the thermal energy through the tissue and the amount of hyalinized collagen is higher in comparison to cold burns. Furthermore, there are important cellular and humoral mediators involved in the pathophysiology of cold burns, as reported in a recent literature review.[21] Therefore, this scenario is able to change the optical properties of the burned skin, as already verified in the literature.[22]

Thus, the photons may have been so attenuated within the tissue that the radiant exposure of 1 J/cm^2 was not sufficient to accelerate the inflammatory stage of the repair process and, consequently, did not present collagen fibers as organized as the 1% silver sulfadiazine group.

Conclusion

Morphologically, the treatments using laser or silver sulfadiazine were similar and both provided greater organization of the collagen fibers compared to the untreated group. However, under the parameters used in this study, SG modulated the deposition of collagen fibers more efficiently than the laser group. There was a systemic effect in the group that received photobiomodulation when the presence of granulation tissue was analyzed.

Resumo

Queimadura experimental: comparativo entre sulfadiazina de prata e fotobiomodulação

Objetivo: Analisar características morfológicas e organização das fibras colágenas de queimaduras de terceiro grau provocadas por escaldo em relação à terapia com *laser* e àquela considerada padrão-ouro, a sulfadiazina de prata.
Método: Foram selecionados 12 animais (*Rattus norvegicus*), divididos igualmente em três grupos (grupo controle [GC] – queimaduras não tratadas; grupo sulfadiazina [GS] – queimaduras tratadas com sulfadiazina de prata 1%; grupo *laser* [GL] – queimaduras tratadas com fotobiomodulação). As queimaduras foram realizadas por escaldo com a utilização de molde de PVC, e o material coletado no 14º dia pós-queimadura foi preparado para análise morfológica e de retardo óptico, para avaliação do infiltrado inflamatório e da organização do colágeno, respectivamente.
Resultados: No 14º dia, os grupos *laser* e sulfadiazina apresentaram resposta inflamatória leve, enquanto o grupo controle apresentou processo inflamatório intenso, havendo significância estatística entre os grupos *laser* e controle, mas não entre os grupos sulfadiazina e controle. Enquanto os grupos *laser* e sulfadiazina não apresentavam mais tecido de granulação, o grupo controle ainda apresentava. A presença de folículo piloso e de úlcera não diferiu significantemente entre os grupos. O retardo óptico das fibras colágenas foi maior no grupo sulfadiazina, seguido dos grupos *laser* e controle. Apenas a análise da presença ou ausência de tecido de granulação permitiu identificar o efeito sistêmico.
Conclusão: Morfologicamente, os tratamentos com *laser* ou sulfadiazina de prata foram similares e ambos proporcionaram maior organização das fibras colágenas em relação ao grupo não tratado. Entretanto, o grupo sulfadiazina modulou a deposição das fibras colágenas mais eficientemente que o grupo *laser*.

Palavras-chave: queimaduras, escaldo, sulfadiazina, *laser*, colágeno, ratos.

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Factors related to non-adherence to mammography in a city of the Brazilian Amazonian area: A population-based study

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Summary

Objective: To assess the prevalence of mammography use and factors related to non-adherence in Boa Vista, capital of Roraima, Brazil.
Method: A cross sectional study, quantitative analysis, based on household survey was performed between June and August 2013, using a face-to-face interview with a pre-tested form. Target population was women between 40 and 69 years. The sample size target was 240 participants, and the sampling method was random cluster sampling. The study was approved by the Institutional Review Board of Federal University of Roraima.
Results: 241 women were included without refusals. The prevalence of non-use of mammography in the past two years was 55.6% (95CI 49.1–61.9). In univariate analysis, the risk factors for non-adherence to mammography were having low educational level, family income below three minimum wages, receiving government assistance, not having consulted with a doctor and no health insurance. In multivariate analysis, only low educational level and receiving government assistance remained as risk factors. Medical consultation or health worker visiting were protective factors.
Conclusion: Adherence to mammography is unsatisfactory in Boa Vista, Roraima, and has a predominantly opportunistic character. Low educational level is confirmed as an independent risk factor, but belonging to a family that receives government assistance can be interpreted as a social marker of families and/or areas lacking of government intervention to increase access to breast cancer control programs.

Keywords: mammography, mass screening, breast neoplasm, health services coverage.

Study conducted by the Graduate Program in Health Sciences, Universidade Federal de Roraima (UFRR), Boa Vista, RR, Brazil

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Introduction

Although the incidence of breast cancer is higher in economically developed countries, higher mortality rates have been registered in underdeveloped countries, such as Brazil.[1,2] There is also a time trend increasing this discrepancy. While a reduction to the order of 11 absolute percentage points in breast cancer-specific mortality has been observed in the United States between 2001 and 2009,[3] the data indicates an increase in mortality from this disease in Brazilian women over the last three decades in almost all age groups, especially younger individuals (up to 50 years of age).[2,4] According to the National Cancer Institute (INCA), in 2014 there were approximately 16 deaths per 100,000 women in Brazil.[2]

Countries and regions that have been able to reduce breast cancer mortality credit this decline to the use of more effective therapies associated with large population screening programs for early detection of the disease. Indeed, the initial extent of the disease is the main predictor of survival in women affected by breast cancer.[5] In countries whose breast cancer screening programs are inefficient or scarce, most women are diagnosed at advanced stages of

the disease and the overall five-year survival rate is relatively low.[6-8] On the other hand, in regions with large scale screening programs a reduction in breast cancer mortality ranging from 15 to 30% has been reported.[7,9,10]

Adherence to mammography-based screening, in turn, suffers multifactorial influences, such as cultural, social and economic issues, public knowledge about the disease and the examination, and the provision of mammography exams in the public and private sectors.[11-14] Despite the control of breast cancer having been included among the priorities of the Brazilian Health Pact,[15] the estimated mammography coverage obtained via household surveys shows insufficient and unequal screening in Brazilian regions.[12,16,17] In a country as heterogenous as Brazil, regional studies can demonstrate variations in the determinant factors of mammography adherence, propitiating adjustments to preventive strategies in accordance with local characteristics. The aim of this study is to assess the prevalence of the use of mammographic screening and the factors related to non-adherence in Boa Vista, the capital of the state of Roraima, in the Brazilian Amazon area.

Method

Study design

This is a cross-sectional, observational study with a quantitative analysis, based on household survey of a random sample in the municipality of Boa Vista, designed to assess women's adherence to breast cancer screening examinations, as well as demographic characteristics, between June and August 2013.

Setting and population of the study

The study was conducted in the municipality of Boa Vista, the capital of the state of Roraima, located within the Legal Amazon, in Northern Brazil. With a population of approximately 285,000 inhabitants, the municipality of Boa Vista concentrates approximately 65% of the state population, and has a Family Health Program (PSF, in the Portuguese acronym) that covers 75% of its population. The target population of the survey was women between 40 and 69 years of age resident in the municipality of Boa Vista for at least two years.

Sampling

The sample size was calculated considering the expected prevalence of 60% coverage for cancer screening, based on a national telephone survey conducted in 2008,[18] assuming a normal distribution for the desired confidence interval of 95% and an acceptable error of 5% (±2.5%), leading to a sample size of at least 240 individuals.

The sampling method was randomized by conglomerate, considering city blocks as the sample conglomerates. There are 4,902 blocks that make up the districts in the urban zone of the municipality of Boa Vista. These were listed and drawn by software, producing a random number sequence (http://www.random.org). The random selection of the blocks was weighted by population size and the number of registered households in each epidemiological zone of the municipality.

Research procedures

All households in the first 25 blocks selected were visited, and female residents belonging to the target age group were approached in their homes during the early morning or evenings on weekends, and invited to participate in the study by signing the informed consent form. Data collection took place between June and August 2013.

We included women aged between 40 and 69 years, since the recommendation at the time was to not exclude younger women from screening (40 to 49 years). The study excluded women who were present but did not reside at the household, who were outside of the age range, who had resided in another municipality in the last two years, and those who did not accept participation in the research. If the resident woman was not at home at the time of the visit, the researcher would return to the residence the following week. In the case of a second absence, the woman was excluded. If the sample goal was not reached in the first 25 blocks visited, another five blocks would be drawn, using the same method successively until the sampling goal was achieved.

A semi-structured form was used as a research tool instrument, with open and closed questions, prepared by the authors, and previously tested to evaluate descriptive and explanatory variables such as age, education, marital status, socioeconomic data, government aid, health agent visits, and history of medical visits. The form was answered via a face-to-face interview at the volunteer's home, preferably in the absence of the volunteer's cohabitants, and for a maximum of 30 minutes. The outcome variable assessed was non-adherence to the breast cancer screening program, defined as failure to undergo at least one mammogram in the last two years prior to the date of the interview, regardless of the outcome of the test and where it was conducted.

Quality control

After the completion of the fieldwork, 10% of the forms obtained by each interviewer were selected for quality control. The patients were re-interviewed by the principal in-

vestigator, via telephone, referring to what were considered key questions. Responses to the key questions were compared to those obtained in the first phase of the field research. In the event of disagreement greater than 5% between the responses of at least one volunteer, all observations collected by the stated researcher were to be discarded.

Statistical analysis

A descriptive statistical analysis was performed, including the frequency distribution for categorical variables, and the means (with standard deviation) for continuous variables with normal distribution. The prevalence of the outcome variable (non-adherence to the use of mammography) and its 95% confidence interval (95CI) were estimated based on binomial distribution. For comparison of the sample means, Student's t-test was used for variables with a normal distribution and homogeneity of sample variances. Otherwise, the Mann-Whitney U test was used for this purpose. We used Chi-squared test to compare differences in the proportions of categorical variables. Odds ratio (OR) and 95CI were calculated in a univariate analysis and the adjusted odds ratio (aOR) was calculated in a multivariate analysis by logistic regression. The selection criteria of the explanatory variables for input into the multivariate analysis was a critical value of $p<0.15$ in the univariate analysis. The level of statistical significance was set at 5%. The information was analyzed after double data entry and the databases were compared to detect data entry errors. The statistical analyses were conducted using EpiInfo® software, version 7.1 for Windows (CDC, Atlanta, US).

Ethical aspects

The study was approved by the Committee for Research Ethics involving humans at the Federal University of Roraima (report no. 111,007 of 2013). All of the adult women volunteers approached were fully clarified as to the purposes and methods of the research and signed the consent form before the interview. The documents were coded rather than being identified. At the end of the interview, all the volunteers were given an explanatory folder on breast cancer, the importance of mammography, and the health facility where it can be conducted. The leaflets distributed were purchased from the State Health Department and are part of the publicity material of the Ministry of Health of Brazil.

Results

Two hundred forty-one (241) female residents of the 30 blocks in the municipality of Boa Vista, Roraima, were interviewed, without refusals or loss of data. The average age was 48.3 years (±5.3), and approximately one third of the sample was between 40 and 44 years of age. The marital status reported the most was married/common-law partner (n=127; 52.7%). None of the participants were illiterate, and the most frequent education levels were up to the primary level (n=93; 38.6%) and secondary level (n=93; 38.6%). Most of the women studied did not have private health plans (n=199; 84.0%), but most participants reported having consulted a physician within the past year (n=202; 85.3%). The average household income reported was BRL 2,016 (approximately three minimum wages at the time of the study). Most women had a household income of less than BRL 2,000 (n=155; 64.4%). The average number of family cohabitants was 4.0 (±1.8). Less than half of the participants reported receiving social aid from the government (n=105; 44.3%) or receiving a health agent visit (Family Health Strategy) within the past year (n=59; 24.9%). Table 1 describes the demographic characteristics of the sample.

One hundred forty-eight (148) women of the 241 participants analyzed (61.4%) reported having carried out a mammogram at some point in their life. Only 107 women (44.4%) reported having undergone the examination within the last two years. Therefore, the prevalence of non-use of mammography for breast cancer screening in the past two years was 55.6% (95CI 49.1–61.9). There was a trend towards lower use of mammography in younger women (40 to 49 years) compared with those aged 50 years or more (60.4 *vs.* 49.5%, respectively), without statistical significance.

Marital status was not correlated with greater or lesser adherence to screening with mammography. With regard to education, non-use of the examination was significantly higher for women with a low educational level in relation to those with undergraduate/graduate education. For women who reported education up to primary level, most of them did not undergo the examination (67.8%, p=0.005), generating an OR 2.19 (95CI 1.26–3.81). For those that reported higher education, the prevalence of non-use was limited to 34.5% ($p<0.0001$), reducing the likelihood of non-use to a third (OR 0.32, 95CI 0.17–0.61). The average level of education was not correlated with greater or lesser adherence to breast cancer screening. Table 2 details the results of the univariate analysis.

It should be noted that the reported household income was also correlated with adherence to the preventive examination in the univariate analysis. Women with a reported income of less than BRL 2,000 (three minimum wages at the time of the study) showed higher prevalence of non-use of mammography than those with an income of more than BRL 4,000 (65.8 vs. 27.9%, respectively; $p<0.0001$). In this analysis, income of less than three minimum wages

more than tripled the chance of not having undertaken a mammogram in the previous year (OR 3.24, 95CI 1.87–5.62). Having private health insurance was shown to be a protective factor. Non-use of the exam was two times lower in women insured by a health plan than in uninsured women (28.9 vs. 60.6%, respectively, p=0.0006; OR 0.26, 95CI 0.12–0.56). On the other hand, receiving government aid (social benefits) represented a risk factor for non-adherence to breast cancer screening (OR 3.46, 95CI 2.01–5.96). Attending a medical consultation (for another reason) over the past year represented an important protective factor, substantially reducing the chance of non-adherence to mammography (OR 0.16, 95CI 0.06–0.43). It should be noted that 86% of the participants who did not report a medical consultation in the past two years also did not undertake a mammogram during that period. Having received a visit by a health agent and number of cohabiting family members were not correlated with the outcome studied (Table 2).

The variables that presented a significant correlation or tendency to correlate with the outcome in the univariate analysis (p<0.15) were reevaluated in a multivariate analysis in order to detect confounding factors. In this analysis, the only variables that remained risk factors for non-adherence to breast cancer screening were low level of education and receiving government aid. Women with education restricted to the primary level presented a likelihood of non-use of mammography that was almost twice as high (adjusted OR 1.98, 95CI 1.48–3.05) in relation to women with a higher level of education. Receiving government aid also doubled the chance of non-use of mammography (adjusted OR 2.27, 95CI 1.14–4.52). Consulting a physician in the past year (adjusted OR 0.16, 95CI 0.05–0.46) and receiving health agent visits (adjusted OR 0.43, 95CI 0.22–0.85) were confirmed as protective factors, both significantly reducing the chance of not undertaking a mammogram. Age, income, and health plan variables were not sustained as independent risk factors in the multivariate analysis (Table 3).

Discussion

Although occasionally recommended by national and international guidelines, early detection strategies other than mammography fail to demonstrate effectiveness in reducing breast cancer mortality. Breast self-examination is not advocated by INCA and other organizations as there is no evidence of this measure's benefits in terms of reducing mortality.[19,20] Clinical examination of the breast is recommended because it is a part of medical semiology. However, it has substantially less diagnostic accuracy than mammography for early breast cancer investigation, and no impact on the reduction of mortality.[21,22] For these reasons, information on self-examination or clinical breast examination were omitted from our study.

Mammography is the only strategy capable of reducing breast cancer mortality (estimated decrease of 30%) in women over 50 years of age regularly screened every 24 months.[22-24] For younger women (< 50 years), the effectiveness of mammograms is controversial because it is related more with an increase in costs from unnecessary interventions than with reduced mortality.[25] During the agreement of the goals for the control of breast cancer in 2006, the Ministry of Health of Brazil recommended a biennial mammogram for women aged 50 to 69 years and an annual clinical breast examination for those aged between 40 and 49 years.[15] However, Law N. 11.664/2008 assured mammograms for all women aged over 40 years.[26] In November 2013, after the collection of data in this survey, Ministry of Health Ordinance N. 1253 modified access,[27] maintaining the guarantee of screening mammography only for women between 50 and 69 years of age, limiting mammography to unilateral diagnosis in women aged between 40 and 49 years.

The present study was a pioneer in the assessment of factors related to the use of mammographic investigation for screening for early breast cancer in the North region of Brazil. In our study, the prevalence of non-adherence was 55.6%, which is higher than the non-adherence rates observed in other household surveys in Brazil. Marchi et al.[28] conducted a study that surveyed 460 women in the city of Taubaté, São Paulo, served at public and private health services in 2010. The authors reported a prevalence of non-adherence to mammography of 32% in the last 24 months. Another similar study,[29] conducted in Pelotas, Rio Grande do Sul, analyzed 879 women aged 40 to 69 years, and revealed that 30% of those interviewed had not undergone a mammogram (in the last two years). The coverage assessed by our study was shown to be heterogeneous and influenced by socioeconomic factors. We found that women with a higher level of education presented a greater chance of undergoing mammography: attaining the secondary level of schooling led to a 15% gain. Higher education produced a gain of more than 30% compared to primary level. Something similar was reported by Oliveira et al., who analyzed data from National Household Sample Survey – PNAD 2003 and 2008.[30] In their study assessing secondary data, albeit nationwide, 54.6% of women aged 50 to 69 years reported having undergone mammograms in 2003, and 71.5% in 2008. The chance of conducting an examination increased with

household income and level of education. The authors reported that having more than ten years of study tripled the chance of conducting a mammogram compared to those not formally educated.

Factors related to non-adherence also vary among the regions studied. In a study by Sclowitz et al.[29] (Pelotas, 2005), the factors related the most to not undertaking mammograms were low social class, lack of family history of breast cancer, and not having had a gynecological consultation in the period assessed. In a study by Oliveira et al.,[30] the risk factors highlighted included age over 70 years, being single, having a low income, not having health insurance, not having carried out consultations with a physician in the past 12 months, living in rural areas or in the North Region of the country. Meanwhile, in a study by Marchi et al.[28] (Taubaté, 2010), the factors related to non-adherence included being an exclusive user of the SUS (Brazilian public health system), irregular gynecological consultations, and never having undergone a previous mammogram. Corroborating the findings of our study, it can be seen that having a consultation with a physician is one of the main protective factors for undergoing a mammogram, suggesting that breast cancer screening is still mostly opportunistic in Brazil. The influence of medical

TABLE 1 Demographic characteristics of the sample studied (n=241).

Variable	Average (±SD)	n (%)
Age (years)	48.3 (±5.3)	
40 to 44 years		78 (32.4%)
45 to 49 years		56 (23.2%)
50 to 54 years		71 (29.5%)
≥ 55 years		36 (14.9%)
Marital status		
Single		67 (27.8%)
Married/common-law partner		127 (52.7%)
Divorced/separated/widowed		47 (19.5%)
Education		
Illiterate		0
Primary school		93 (38.6%)
High school		93 (38.6%)
Higher education/postgraduate		55 (22.8%)
Has insurance/health plan		
Yes		38 (16.0%)
No		199 (84.0%)
Household income (BRL)	2,016.24 (±2,145.03)	
< BRL 2,000		155 (64.4%)
Between BRL 2,000 and BRL 4,000		51 (21.1%)
> BRL 4,000		35 (14.5%)
Cohabiting family members (n)	4.0 (±1.8)	
> 4 family members		81 (34.2%)
Up to 4 family members		156 (65.8%)
Government aid		
Receiving		105 (44.3%)
Not receiving		132 (55.7%)
Medical consultation in the past year		
Yes		202 (85.3%)
No		35 (14.7%)
Health agent visit		
Yes		59 (24.9%)
No		178 (75.1%)

TABLE 2 Univariate analysis for evaluation of non-adherence to mammographic screening over the last two years in the municipality of Boa Vista, Roraima, 2013.

Explanatory variable	Total	MMG not conducted in the last 2 years		p-value	Odds ratio	95CI
		n	%			
Age						
Between 40 and 49 years	134	81	60.4	ns	1.57	0.93–2.60
50 years or older	107	53	49.5		1	
Marital status						
Single	67	41	61.2	ns	1.37	0.77–2.44
Married	127	66	51.9	ns	0.73	0.43–1.22
Widowed/divorced	47	27	57.5	ns	1.09	0.57–2.08
Education						
University	55	19	34.5	<0.0001	0.32	0.17–0.61
High school	93	59	53.7	ns	0.88	0.52–1.49
Primary school	84	57	67.8	0.005	2.19	1.26–3.81
Household income						
< BRL 2,000	155	102	65.8	<0.0001	3.24	1.87–5.62
Between BRL 2,000 and 4,000	43	20	46.5	ns	0.64	0.33–1.24
> BRL 4,000	43	12	27.9	0.0001	0.24	0.11–0.49
Has health insurance						
Yes	38	11	28.9	0.0006	0.26	0.12–0.56
No	203	123	60.6		1	
Cohabiting family members						
> 4 family members	83	53	63.8	ns	1.67	0.97–2.89
Up to 4 family members	158	81	51.2		1	
Government aid						
Receiving	107	77	71.9	<0.0001	3.46	2.01–5.96
Not receiving	134	57	42.5		1	
Medical consultation						
Yes	205	103	50.2	0.0001	0.16	0.06–0.43
No	36	31	86.1		1	
Health agent visit						
Yes	61	28	45.9	ns	0.59	0.33–1.06
No	180	106	58.9		1	

MMG: mammogram; ns: not significant (p-value > 0.05); 95CI: 95% confidence interval.

TABLE 3 Multivariate analysis for non-adherence to mammographic screening in the municipality of Boa Vista, Roraima, 2013.

Variable	Adjusted odds ratio	95CI	p-value
Age between 40 and 49 years	1.64	0.90–2.99	ns
Primary school only	1.98	1.48–3.05	0.008
Higher education	0.96	0.39–2.34	ns
Household income less than BRL 2,000	1.33	0.55–3.22	ns
Household income higher than BRL 4,000	0.49	0.18–1.35	ns
Having health insurance	0.52	0.20–1.32	ns
Receiving government aid	2.27	1.14–4.52	0.01
Having a medical consultation in the past year	0.16	0.05–0.46	0.0007
Having been visited by a health agent	0.43	0.22–0.85	0.01

ns: not significant (p-value > 0.05); 95CI: 95% confidence interval.

advice has also been reported in other countries. A cross-sectional study conducted in the United States[31] in the year 2000 and involving 1,301 women found that medical advice is the variable most strongly associated with the use of mammography. Women who reported medical advice were more likely to adhere to breast cancer screening. The impact of medical advice was so important that the authors postulated a model of increased use of mammography in two stages: first, public call; and secondly, individual encouragement by the physician. Another North American study[32] specifically assessed the importance of medical communication on adherence to mammography among 972 women over 50 years of age. A 4.5 times greater propensity to performing mammography was reported among women who received encouragement from their physicians with respect to the benefits of the examination. The authors concluded that the key to increasing the coverage of breast cancer screening is improved communication skills between physicians and patients.

It is interesting to note that a visit from a health care agent was also correlated with adherence to mammography in our study, highlighting the importance of professional advice in the promotion of health in this sample. We noted that among women who had not consulted a physician in the past two years, the prevalence of non-adherence was very high, at 86%. This indicates two main scenarios: difficult access to the examination by spontaneous demand and poor public knowledge about the importance of the examination, which may have been minimized via medical consultations in the period. A survey that assessed women's knowledge about the subject[33] (São Paulo, 2011) revealed that the subject of "breast cancer" is well-known to women, but mammography still needs to be clarified in relation to its objectives and recommendations, representing a possible barrier to satisfactory coverage of the population screening.

Another important fact was the demonstration of education level as an independent determinant of the use of mammography, overlapping with income in the multivariate analysis. Although the study by Sclowitz et al.[29] found a correlation between low social class and non-performance of the examination, the authors' evaluation did not include the numerous characteristics defining social class. In our study, reaching secondary education attributed a gain of 15%, while higher education led to a gain of 30% compared to women with primary educational level, meaning that more years of schooling was confirmed as an independent protection factor. In relation to the receipt of social benefits, despite the correlation not being statistically significant in the univariate analysis, this variable was established as an independent risk factor in the multivariate analysis. Although this variable might at first be interpreted merely as a confounding factor, it should be analyzed as a social marker, as it represents easily identified registered families, assisting health managers in the zoning of areas most in need of government intervention.

This study has limitations. Firstly, the conglomerate sampling method used might fail to make the sample accurately representative of the population studied. Secondly, the cross-sectional design presupposes not allowing the use of temporality as a criterion for causality, given that the risk factors and outcome were measured at the same time and the bias of reverse causality cannot be eliminated. Finally, studies based on face-to-face interviews are susceptible to the masking of answers, especially with the subject of personal health. However, the sample size and the robustness of the research procedures adopted strengthen the reliability of the data.

Conclusion

The coverage of breast cancer screening using mammography is unsatisfactory in Boa Vista (RR), and has an opportunistic character because of its correlation with a background of medical consultations and health agent visits over the last two years. A low level of educational is confirmed as an independent risk factor for non-adherence to screening, and belonging to a family that receives government aid can be interpreted as a social marker of families and/or areas lacking government intervention in order to increase access to breast cancer control programs.

Conflict of interest

The authors declare no conflict of interest.

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Resumo

Fatores relacionados à não utilização de mamografia em capital brasileira da região Norte: um estudo de base populacional

Objetivo: Avaliar a prevalência de utilização da mamografia e fatores relacionados à não adesão em Boa Vista, capital de Roraima, Brasil.

Método: Trata-se de um estudo de corte transversal, de análise quantitativa, baseado em inquérito domiciliar, por entrevista face a face, utilizando formulário previamente testado. Foram incluídas mulheres entre 40 e 69 anos, entre junho e agosto de 2013. A meta amostral foram 240 participantes, e o método de amostragem foi aleatório por conglomerado. O estudo foi aprovado pelo Comitê de Ética em Pesquisa da Universidade Federal de Roraima.
Resultados: 241 mulheres foram incluídas, sem recusas. A prevalência de não utilização de mamografia nos últimos dois anos foi 55,6% (IC95% 49.1–61.9). Em análise univariada, os fatores de risco para não adesão à mamografia foram baixa escolaridade, renda familiar inferior a três salários mínimos, receber auxílio governamental, não ter sido consultado por médico e não ter plano de saúde. Em análise multivariada, apenas baixa escolaridade e receber auxílio governamental se mantiveram como fatores de risco, enquanto consulta médica ou visita de agente de saúde, como fatores de proteção independentes.
Conclusão: A adesão à mamografia é insatisfatória em Boa Vista e tem caráter predominantemente oportunista. Baixa escolaridade se confirma como fator de risco independente, mas pertencer a uma família que recebe auxílio governamental pode ser interpretado como marcador social das famílias e/ou áreas mais carentes de intervenção governamental para aumentar o acesso aos programas de controle do câncer de mama.

Palavras-chave: mamografia, programas de rastreamento, neoplasias de mama, cobertura dos serviços de saúde.

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The P-A-C-I-E-N-T-E Protocol: An instrument for breaking bad news adapted to the Brazilian medical reality

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Summary

Objective: There are plenty of published tools for breaking bad medical news; however, none of them is culturally appropriate to our reality or published in the Brazilian literature. This study proposes a genuinely Brazilian communication tool and evaluates its acceptance among doctors and nurses.
Method: This was a prospective study. The data were collected after specific training of doctors and nurses on the bad news communication techniques based on the P-A-C-I-E-N-T-E ("patient," in Portuguese) Protocol. This instrument is in accordance with the Brazilian reality and was based on the SPIKES communication tool.
Results: The worst task to be performed during communication is "talking about death" followed by "discussing the end of curative treatment attempts" and "diagnosis" itself. Among the respondents, 48% reported they did not receive formal training for communicating. Also, 52% of respondents do not use any systematic approach in their daily practice when communicating with patients, but 97% considered the proposed P-A-C-I-E-N-T-E Protocol as a useful and appropriate communication tool.
Conclusion: The P-A-C-I-E-N-T-E Protocol proved to be suitable to the Brazilian context.

Keywords: palliative care, protocol, communication.

Introduction

Breaking bad news to a patient can be considered as one of the moments of greatest anxiety in medical practice.[1-3] Communication protocols are intended to minimize this stress, facilitating the development and maintenance of a good doctor-patient relationship.[4-7]

Bad news can be defined as the revelation of a diagnosis of a potentially life-threatening disease, such as cancer, as well as the failure of a curative therapy, but also include the discussion of a poor prognosis and the proximity of death.[1,4,6] According to the Brazilian Code of Medical Ethics (Chapter V, art. 34),[8] breaking bad news is a medical act that should not be delegated. However, nurses are professionals that are very present in patient care, and will often repeat and explain what was said by the doctors. These professionals also have their repertoire of bad news to communicate to the patient, such as the need for a new venous access to be punctured.[9]

The way that bad news is transmitted generates a result that is more harmful to the patient and their family than actual content informed.[4,5] Thus, specific communication protocols and training can encourage the establishment of empathy and trust between the patient and his/her doctor.

There are communication protocols proposed in the international literature that are effective in reducing the stress of professionals as well as facilitating the process of informing patients.[4,6,10-13] However, there are no genuinely Brazilian protocols proposed in the literature, or even protocols that have been adapted.

The cultural and ethnic specificities of our country evidently require a different type of communication or approach from those already existing.

The main objective of our study was to propose a communication protocol in Brazilian Portuguese that is easily memorable and culturally adapted to the Bra-

zilian reality, evaluating its acceptance among doctors and nurses.

It also aims to evaluate the perceptions of nurses and doctors about the process of breaking bad news.

Method

After approval by the Institutional Research Ethics Committee and obtaining informed consent, 226 questionnaires were applied to higher level health professionals (doctors and nurses). This is a prospective study conducted at a single research center, where the data was collected over a period of two years, after specific training of health professionals in communication skills for breaking bad news and the use of an instrument to facilitate the process. This instrument is based on a model adapted from the SPIKES Protocol, using a mnemonic code adapted to Brazilian reality, called the P-A-C-I-E-N-T-E Protocol (Chart 1). The evaluation of the appropriateness and usefulness of the protocol was based on the opinion of the participants in the study, as presented in the research questionnaire.

In addition to the six steps transcribed by SPIKES, in the adapted protocol there is a seventh stage, namely: "Don't abandon the patient," which is paramount to meeting patients' expectations. Patients are often afraid of death and how it will occur, resulting in fear of being abandoned.[14] The training sessions were conducted by the authors during realization of scientific events promoted by several medical societies (the Brazilian Society for Study of Pain – SBED; the Brazilian Society of Anesthesiology – SBA; and the Society of Anesthesiology in the State of São Paulo – SAESP) in an itinerant manner.

After being submitted to the training, which was characterized by a formal expository lesson on communicating bad news and presentation of the P-A-C-I-E-N-T-E Protocol, followed by a practical "role play" activity, doctors and nurses of any specialty were invited to participate in the study. There was no limitation of participants in relation to sex or age.

Only questionnaires that were filled out incompletely or were unreadable were excluded from the sample, leading to the total exclusion of 26 questionnaires. Participation was free, and included all participants of the training sessions, as there was no refusal to participate.

The training offered initially consisted of a brief introduction about the importance of breaking bad news in the practice of health professionals. The P-A-C-I-E-N-T-E Protocol, a mnemonic information method consisting of seven steps, was presented as described below.

P – Prepare

Health professionals should be prepared before transmitting bad news appropriately. First, the veracity of the information to be revealed must be confirmed by consulting the medical record. It is also recommended to consult the medical literature in order for any possible doubts to be resolved. It is necessary to prepare the environment properly, ensuring total privacy and comfort. Preferably, there should be no physical barriers standing between the doctor and the patient. The professional should ensure that no unexpected interruptions will occur during communication and should sit at the same height as the patient.

A – Assess how much the patient knows and how much they want to know

It is important to assess the patient's level of knowledge about their diagnosis. Similarly, question what level of information the patient would like to receive at this time, or if they actually do not wish to be informed of their diagnosis. In this case, the patient may indicate someone they trust to receive the information on their behalf.

P – Prepare
A – Assess how much the patient knows and how much they want to know
C – (*Convite à verdade*, in Portuguese) Invite the patient to the truth
I – Inform
E – Emotions
N – (*Não abandone o paciente*, in Portuguese) Do not abandon the patient
TE – (*Trace uma estratégia*, in Portuguese) Outline a strategy

CHART 1 Definition of the steps of the communication protocol that is composed, in a mnemonic way, by the word P-A-C-I-E-N-T-E.

C – Invite the patient to the truth

In this step the patient is informed of the existence of bad news. Use phrases such as: "I'm sorry, but I believe I don't have good news." The patient is thereby offered the possibility of changing their mind as to whether they want to be informed or not. In some situations, the patient may be quiet and not continue beyond the "Invite the patient to the truth" stage. This attitude may indicate that the patient needs more time to understand and work out what they were told.

I – Inform

The best strategy is to wait for the time required by the patient and offer space for them to "invite" the doctor to share the information and ask directly about their diagnosis, prognosis or results.[4-6] The relevant information about the state of the patient's health can then be shared at a sufficient amount, speed, and quality, and at the desired amount, so that the patient can make decisions about their life or offer informed consent about their treatment. Avoid a precise report of the prognosis, as doctors tend to overestimate life expectancy. Offer information clearly and honestly, trying to keep the patient's hopes up while being realistic as to treatment options. Do not use euphemisms but choose the right keywords, such as "cancer" and "metastasis," explaining their significance.[3-6,15,16]

E – Emotions

After the information has been revealed, the patient needs time to understand and react to the bad news. Keep tissues nearby. Allow patients to express themselves. Use touch as a form of communication and comfort. Clarify the patient's doubts, so that they feel accepted and protected.

N – Do not abandon the patient

Ensure that your patient will receive medical monitoring. Make a commitment not to abandon them, regardless of the outcome.

T and E – Outline a strategy

Plan the care to be offered and treatment options with the patient. Include interdisciplinary care in the plan, whenever possible. Request monitoring by other doctors who can assist in the control of symptoms.[3-6,15-18]

Statistical analysis

For the statistical analysis, we used the SPSS Statistics software version 17.0.0. For comparison of the ages in relation to sex and profession, we used Student's t-test. To study the association between the variables, ages were divided into four groups (A = 30 years; B = 30 to 39 years; C = 40 to 49 years; and D ≥ 50 years). Fisher's exact test and Chi-square test were used in relation to these associations. The level of significance adopted was 5%.

Results

Nine out of the total number of questionnaires applied were excluded due to illegibility or completion errors, and 17 due to incomplete data. The collection was interrupted when 100 valid questionnaires from doctors and 100 from nurses were obtained. Nurses were predominantly female (90 participants, $p<0.05$), with no difference between the sexes in the group of doctors. In all included subjects, however, the female sex represented 72% of the sample ($p<0.05$). There was a homogeneous distribution of age groups, but a discreet predominance of those between 30 and 39 years of age, which accounted for 37% of the sample, and is not statistically significant.

For 39.5% of respondents, the most difficult task to be performed during the communication is talking about death. However, 30.5 and 13% found it more difficult to discuss ending attempts at curative treatment and the diagnosis itself, respectively. The following tasks were considered in order of decreasing difficulty: notifying the recurrence of the disease, discussing the diagnosis, and involving the patient's family in the discussion.

When comparing the influence of the professional's gender on the difficulty encountered in the implementation of the tasks there was a coincidence, between men and women, as far as difficulty in discussing death. However, the second greatest difficulty for women was talking about the diagnosis, while for men it was talking about the end of the attempts at curative treatments ($p<0.05$). Age or profession did not affect the perception of difficulties.

Forty-eight percent (48%) of respondents reported not having received formal training on communication, while only 14% had specific lessons on the subject. However, 19.5% of the sample reported having improved their communication skills by observing other professionals and also attending lessons, while 18.5% had been exclusively trained by observing other professionals. There is no statistically significant difference between the age, sex or profession of the participants in this regard ($p>0.05$).

After completion of training with the P-A-C-I-E-N-T-E method, 49% of the individuals believed their ability to communicate bad news was reasonable, classified in descending order as good (25.5%), poor (12.5%), very good (7.5%), and very poor (5.5%). In this matter, there were no statistical differences between gender and age, although there was a statistical difference when comparing profes-

sion. Only 10% of doctors assessed their ability to break bad news as "poor" or "very poor," while 26% of nurses considered their ability likewise (Figure 1).

Many difficulties are encountered when breaking bad news. The most important among those suggested in the questionnaire, according to 42.5% of participants, was "dealing with the patient's emotions," while "being honest without giving up hope" appeared in second place in order of difficulty, with 37.5%. When gender differences are taken into account, male participants present greater difficulty in being honest and maintaining hope, while female participants have greater difficulty in dealing with emotions (Figure 2).

Doctors considered that the easiest step in the P-A-C-I-E-N-T-E Protocol is "Prepare" (38%), while nurses consider it is "Do not abandon the patient" (47%). It is striking that none of the doctors considered the task "outlining a strategy" as the easiest. In relation to age, statistical differences were noted: younger groups have greater ease with "Do not abandon the patient;" the 40-49 years age group, with "Prepare," and the group over 50 years, with "Emotions."

Fifty-two percent (52%) of the participants do not use any systematic approach in their daily practice when communicating with patients. Only 18% use an instrument; and 30% employ several techniques, albeit without a general plan. Surprisingly, approximately 97% of participants consider that the P-A-C-I-E-N-T-E Protocol is appropriate and useful in communicating bad news, according to the survey. These aspects were not influenced by gender, age or profession.

Discussion

It is known that one of the factors that most interferes in the communication of bad news to patients is the health professional's stress, which is usually related to a lack of specific training.[19,20-23]

Most people, regardless of their own cultural characteristics, would like to be aware of their diagnosis, even if it means accepting that they are terminally ill.[6,11,13,24-26] This information is important to patients, as they start to show a greater degree of adaptation to reality, with lower levels of depression and anxiety, better adherence to treatment, more acceptance of interventions, and suitable monitoring of dosages and recommendations[9,27] as well as preventing barriers between the family and the patient (conspiracy of silence).[28] Furthermore, it prevents the patient receiving futile treatments and heroic interventions, creating false hopes and expectations in treatments.[17,20,25,27,29-34]

In Brazil, communication strategies are beginning to gain strength, but a protocol adapted to the Brazilian context has not yet been described. In accordance with

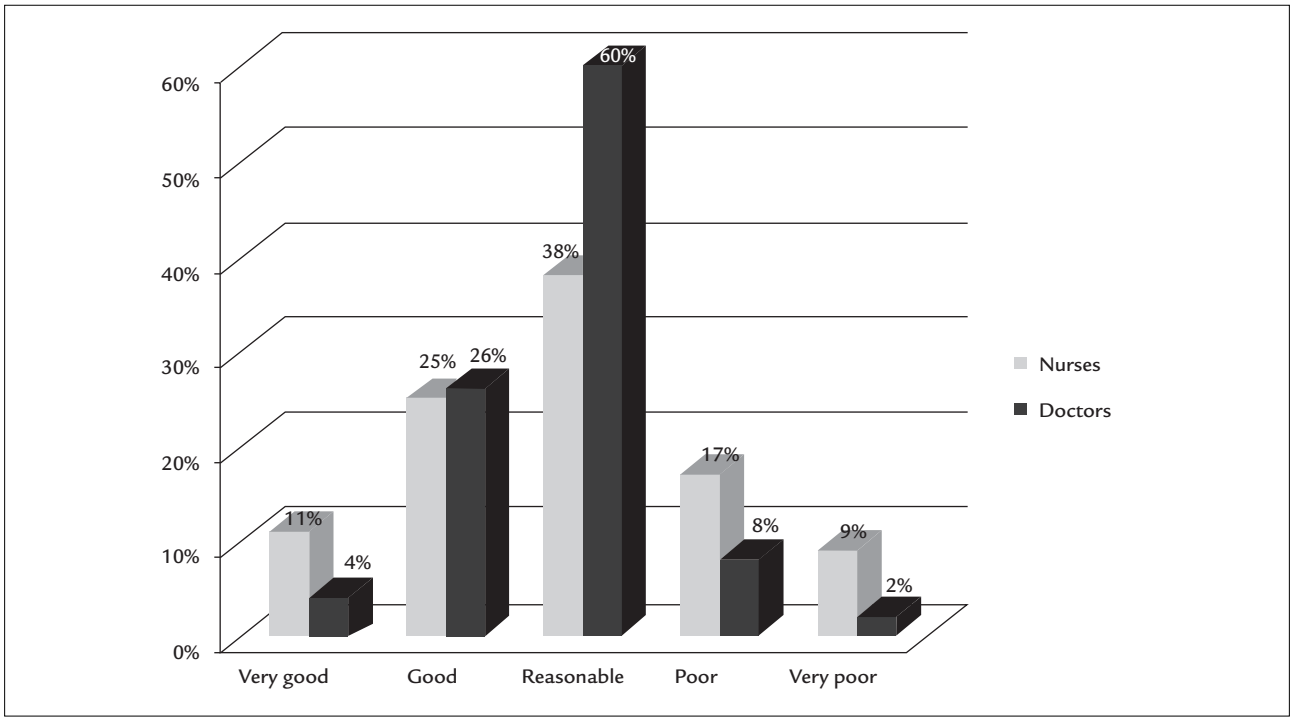

FIGURE 1 Perception of doctors and nurses on their ability to break bad news.

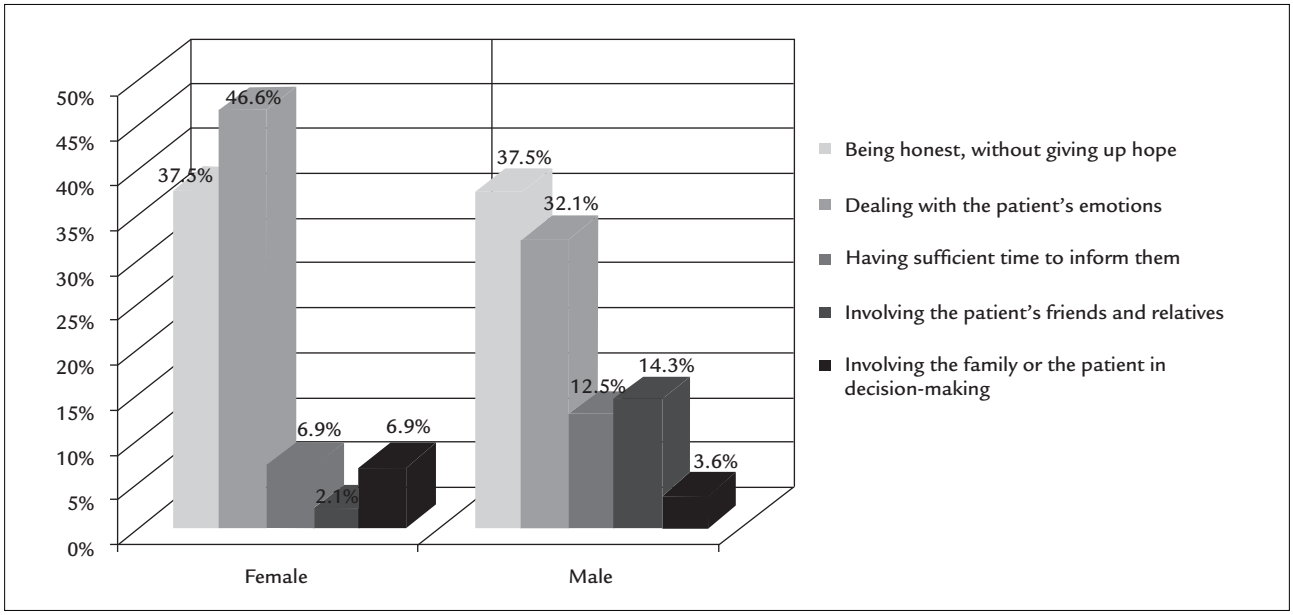

FIGURE 2 Differences in difficulties discussing bad news with patients between the genders of the participants.

this shortfall, we have proposed a protocol for breaking bad news based on the medical literature and in line with the cultural peculiarities of Brazil.[4,7,11-13] This method is based on a mnemonic device using a word in Portuguese ("patient") to facilitate its use in the Brazilian context (Chart 1). Its objective is to facilitate diagnostic and prognostic information in a systematic and truthful manner, respecting autonomy, individuality, Brazilian culture, and the maintenance of hope.

In our study, the female gender was prevalent, especially among the group of nurses. Similar results to ours were found in an Iranian study published in 2010, involving the participation of 50 doctors and 50 nurses.[23]

In our study, 49% of respondents assessed their communication skills as being reasonable, and 18% as poor or very poor. Doctors presented better levels of confidence in their skills than nurses. The results of Arbabi (2010) regarding the self-reported ability of professionals in breaking bad news are equal to ours, that is, 40% of the doctors assessed their ability at disclosing bad news as "good and very good" and 22% as "weak and very weak," data similar to those of Buckman (2000).[4,23] In our study, nurses evaluated their communication skills as worse when compared to doctors, with similar results found in the literature.[23]

In our sample, 44% of respondents had never received any type of training and, among those trained, 22.5% learned exclusively through observation of other professionals. Interestingly, a study published in the United Kingdom showed similar figures: 49% of doctors had never received formal training, although most believe it to be useful in a clinical context.[35] In general, as verified in our context, studies found a low percentage of professionals who had been trained in communicating bad news during their education.[4,23]

In line with the literature, there is a growing concern in changing the educational profile to include communication in the curricula.[19,22] The Accreditation Council for Medical Education in the United States has already included communication with patients as a fundamental skill in the curriculum of residents and graduate students.[10] Considering that, in our study, the percentage of professionals who received communication training does not present a significant difference in relation to the age of the interviewees, we can infer that there has been no important modification in teaching communication at medical and nursing schools in recent decades.

However, most of the health professionals interviewed in several studies believe that training on breaking bad news is important and has the potential of bringing benefits to their clinical practice.[4,36,37] In our study, 97% of the participants agreed that the P-A-C-I-E-N-T-E method could be useful in their professional practice.

We also assessed the potential difficulties and, consequently, the factors that cause anxiety in the process of breaking bad news. "Discussing death" and "discussing the end of attempts at curative treatment" were considered the most difficult tasks in our study. We therefore noted

that issues related to the lack of possibility of finding a cure and, consequently, issues that are frequent in palliative care represent greater difficulties for health professionals than the disclosure of the diagnosis itself.[18,38]

In relation to the procedure for breaking bad news, dealing with the emotions of patients was the most difficult task indicated by our respondents. Most studies published in different cultural scenarios indicate that one of the difficulties cited and emphasized the most by health professionals in the communication process is, coincidentally, dealing appropriately with the emotions of patients and their families.[4,5,12,19,23]

The participants in our study aged 50 years or older showed less difficulty in dealing with patients' emotions, which points to the fact that greater clinical experience as well as maturity can facilitate moments of interaction with the emotions of patients and their families.[17,23,38]

One of the greatest challenges in the communication process is maintaining hope when bad news is revealed.[3,4,12,15,21] In our study, 37.5% of respondents reported that this was the most difficult task. Certain attitudes and characteristics of doctors favor the preservation of hope among patients. These include the professional remaining up to date, demonstrating confidence, ensuring that patients receive appropriate pain treatment, providing realistic information about the future, providing an opportunity for questions and giving explanations about the disease using appropriate expressions such as "cancer" and "death."[3,24] Knowledge of this fact increases the importance of the step "Outlining a strategy" in the proposed protocol.

Interestingly, unlike in other cultures,[24] only a small percentage of the professionals we interviewed mentioned some difficulty or barrier in relation to family involvement in the process. This result may reflect characteristics of the Brazilian culture, in which family involvement is usual, and the family holds a role not only as a passive companion, but also participates in decision-making and in the demand for information.[13,39,40]

In relation to the seven steps of the protocol, participants judged "do not abandon the patient" as the easiest, followed by "prepare." It is probable that on account of the attributes of the profession of nurse, which brings them closer to the patient and family, most of the nurses mentioned "do not abandon the patient" as the easiest task. Doctors, on the other hand, indicated that "prepare" is the easiest task. This is possibly due to the fact that they have unrestricted access to the data needed, and all the theoretical knowledge related to the illness in question.

Generally, when bad news are given by a doctor, the patient needs time to digest what was said, or needs simpler vocabulary to understand the implications of the bad news. Nurses are the professionals who are present for a longer time providing patient care and will often be the ones to repeat and explain what was said by the doctors to the patient.[41]

This study has certain limitations, such as the fact that the sample was not distributed in a controlled manner, given that the scientific events where the training took place attract people from different regions of the country and from different specialties but who have a particular interest in the subject (treatment of pain and palliative care). It also does not specify the amount of professional experience of the participants. The gain in knowledge and communication skills regarding bad news, as given in the majority of publications on the subject, was only assessed subjectively by the participant, without the application of a formal assessment.[42]

There is a need to encourage the study of the subject in our midst, especially by researching preferences and needs of Brazilian patients.

Conclusion

Most of the literature available on this subject focuses on medicine and the patient in a European or American context, and is scarce in our country.[10,19,43] This study quantifies and qualifies the preferences and experiences of doctors and nurses in our reality.

Communication should be one of the skills developed in the curricula of health education institutions since the beginning of studies, when clinical knowledge is introduced. The P-A-C-I-E-N-T-E Protocol has been proposed as a tool to direct and facilitate communication and was shown to be practical and useful for the majority of the participants in this study. The health professional's common sense and experience should be considered when using the protocol.

Resumo

Protocolo P-A-C-I-E-N-T-E: instrumento de comunicação de más notícias adaptado à realidade médica brasileira

Objetivo: Existem inúmeros protocolos de comunicação de más notícias; porém, nenhum método na literatura nacional é culturalmente adequado à nossa realidade. Este estudo propõe um método de comunicação adaptado e avalia sua aceitação entre médicos e enfermeiros brasileiros.

Método: Trata-se de um estudo prospectivo cujos dados foram coletados após treinamentos específicos de médicos e enfermeiros sobre as técnicas de comunicação de más notícias. Foi empregado instrumento mnemônico chamado Protocolo P-A-C-I-E-N-T-E. Esse instrumento, em concordância com a realidade brasileira, foi baseado no Protocolo SPIKES de comunicação.

Resultados: Verificou-se, entre os profissionais da saúde, que a pior tarefa a ser executada durante a comunicação é "falar sobre a morte", seguida de "discutir o fim das tentativas de tratamento curativo" e o "diagnóstico" em si. Do total dos entrevistados, 48% relataram não terem recebido treinamento formal sobre comunicações. Verificou-se, ainda, que 52% dos participantes não utilizam qualquer abordagem sistematizada na prática diária ao se comunicarem com os pacientes, mas 97% consideraram o protocolo proposto útil e adequado.

Conclusão: O Protocolo P-A-C-I-E-N-T-E, proposto como ferramenta para direcionar a comunicação, mostrou-se adequado à nossa realidade.

Palavras-chave: cuidados paliativos, protocolo, comunicação.

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Anticoagulation in acute ischemic stroke: A systematic search

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Summary

Introduction: Stroke is one of the most important diseases worldwide. Several clinical scenarios demand full dose of anticoagulants primary to stroke etiology or to the treatment of comorbidity. However, controversy exists over many issues regarding anticoagulation treatment in stroke such as time for initiation, efficacy according to stroke etiology, the ideal dose of anticoagulants, and whether novel anticoagulants should be used.

Method: Computerized search for clinical trials and randomized controlled clinical trials was done to the present date at Medline, Scielo, Embase, PsychInfo, and Cochrane Library using MeSH terms and the keywords stroke, ischemic stroke, anticoagulation, anticoagulants, heparin, low-molecular-weight heparin, warfarin, dabigatran, rivaroxaban, apixaban. The PRISMA statement was used to evaluate clinical trials.

Results: Fourteen clinical trials were selected based on inclusion criteria. No evidence was found supporting the early use of heparin, heparinoids or low--molecular-weight heparin (LMWH) early after stroke. No consistent evidence for the use of warfarin and the newer oral anticoagulants were found. Argatroban was the only anticoagulant with significant positive results early after large-artery ischemic stroke.

Conclusion: The ideal time for initiating anticoagulation remains undefined, requiring further investigation. Early anticoagulation for ischemic stroke is not recommended, with few exceptions, such as that of argatroban.

Keywords: acute ischemic stroke, anticoagulation, heparin, warfarin.

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Introduction

Stroke is the second most common cause of mortality and the third cause of disability worldwide. Ischemic stroke leads to 2.9 million deaths and to disabling sequelae in approximately 3.4 million patients globally.[1-3] Among all types of stroke, ischemic stroke accounts for 68% and hemorrhagic stroke for 32% of the cases, having a higher incidence in less developed countries.[4] With regard to the etiology of stroke, 20% of cases are due to intracranial large-artery atherosclerotic disease, 25% to lacunar infarctions, 20% to cardioembolism, and 5% to rare etiologies, while 30% are cryptogenic.[5]

Aspirin, antihypertensives, and statins are the most commonly prescribed drugs for secondary prevention of stroke.[6] Anticoagulation therapy is not routinely indicated for acute stroke management owing to the risks of intra or extra-cranial bleeding although it can offer some benefits. Controversy exists over the timing for initiation of anticoagulation, the efficacy of the treatment in relation to stroke etiology, the ideal dose of anticoagulants, and the use of novel anticoagulants.[3,7] Even the form in which the anticoagulant is administered can influence outcomes. For instance, a study with the administration of a bolus of intravenous heparin at doses of 5,000 IU or less did not increase the risk of complications.[8] However, randomized trials involving a larger number of patients to confirm this finding are needed.[8]

Randomized trials further assessing these issues in large patient cohorts have been suggested. However, heterogeneity among studies and different clinical scenarios justifying anticoagulation hamper a meta-analysis of all the clinical studies available.[3]

The objective of the present review is to critically compile the information on anticoagulation after acute ischemic stroke reviewing debatable issues on anticoagulation treatment. We have searched mainly randomized clinical

trials, and other relevant published information in the literature to achieve a higher grade of evidence.

Method

We performed a broad critical review on anticoagulation in acute ischemic stroke. In November 2015, a computerized search for English written clinical trials and randomized controlled trials was done in Medline, using the following MeSH terms and keywords: stroke, ischemic stroke, anticoagulation, anticoagulants, heparin, low-molecular-weight heparin (LMWH), warfarin, dabigatran, rivaroxaban, apixaban. This initial search yielded a maximum of 469 references. A manual search identified studies against an inclusion criteria for eligibility and relevance that incorporated: ischemic stroke, identification of stroke etiology, randomized controlled trials, outcomes clearly identified, report of adverse effects, report of hemorrhage due to anticoagulation, time of initial, and duration of anticoagulation (n=14). The resulting information was supplemented by extensive manual searching of references included at Medline database or in the primary selected studies, especially for prospective, controlled, systematic reviews, and meta-analysis. Other studies reporting safety and tolerability data not reported in prospective or controlled studies were manually searched. The Cochrane Library was searched to assure that this evidence was not systematic reviewed. Also, Embase, Scielo, and PsychInfo databases were searched for relevant studies not reported at Medline, which yielded no additional findings (Figure 1). Finally, eight studies of anticoagulation in acute stroke were selected (Table 1). Study selection was based on the agreement between two authors using the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA; www.prima-statement.org) statement checklist and flow diagram as reference for quality analysis (N.F. and I.A.). Two other authors analyzed the information obtained from the selection of studies (R.M.M. and E.D.N.).

Results

The use of heparin for the acute management of ischemic stroke has been assessed in a number of clinical trials, producing conflicting results (both positive and negative) and thus requiring further evaluation.[9-11]

Table 1 provides a brief summary of the findings of the eight most important clinical trials on anticoagulation in acute stroke. Overall, no clear evidence favoring early anticoagulation was found supporting the use of heparin, heparinoids, LMWH or other agents after acute stroke for the secondary prevention of thromboembolic events, disabling sequelae or death.[12-19] Antiplatelet agents remain the first-choice of antithrombotic agents.

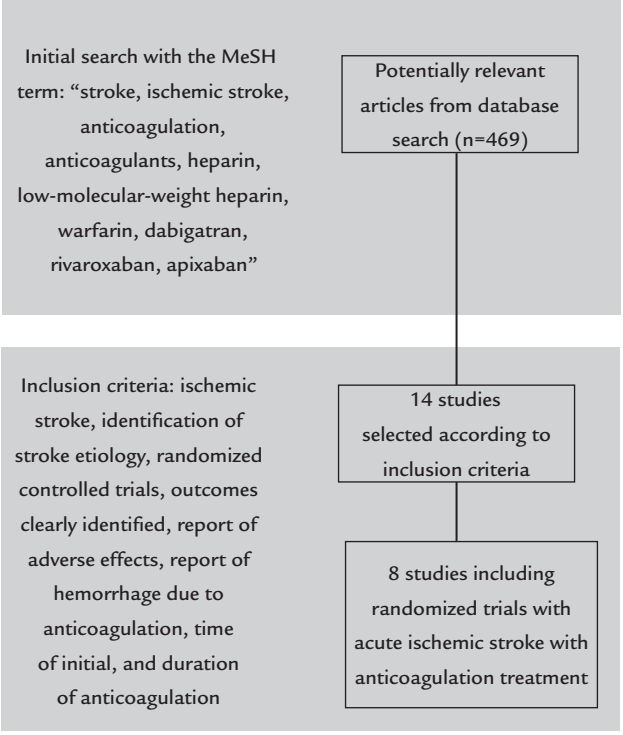

FIGURE 1 Flow diagram of selected studies.

Only two studies showed clinical improvements with anticoagulation.[12,14,20] Kay et al. reported significant dose-dependent reduction in the risk of death or dependency at 6 months with nadroparin.[12] In the TOAST trial there was a significant response observed with anticoagulation using danaparoid at both 7 days and 3 months among subjects with stroke secondary to large-artery atherosclerosis (Table 1).[14] The ARGIS-1 trial used anticoagulation with argatroban safely to subjects with acute stroke also due to large-artery atherosclerosis, but without clinical or functional outcomes between the treatment groups (Table 1).[19]

Non-vitamin K antagonist oral anticoagulants (NOAC) such as dabigatran, rivaroxiban, and apixaban were not properly evaluated in the acute management of ischemic stroke.[20-22] Clinical trials have not included these agents to anticoagulation in the acute stroke phase. Only 44 patients have randomly received apixaban or warfarin within 7 to 14 days of previous stroke in the ARISTOTLE trial, without stroke or systemic embolism in the follow-up and only one major bleed with warfarin.[22]

The main reason to avoid the use of anticoagulants was a variable increase to the risk of bleeding (Table 1). Even at the TOAST trial, a higher bleeding rate was seen in the danaparoid group.[14] However, bleeding rates did not differ between the therapeutic and placebo groups of the ARGIS-1 trial using argatroban.[19]

TABLE 1 Brief description and results of IST, TOAST, HAEST, TAIST, FISS-tris, and ARGIS-1 trials comparing anticoagulation versus aspirin or placebo in the acute management of ischemic stroke (up to 14 days after the event).

	FISS	IST	TOAST	HAEST	TAIST	TOPAS	FISS-tris	ARGIS-1
Drug used	Nadroparin 4,100 IU (aXa), SC, 2x/daily; nadroparin 4,100 IU, once daily, or placebo	UFH 5,000 or 12,500 IU, SC, 2x/daily, with or without aspirin 300 mg	Danaparoid, IV, *in bolus*+ continuous infusion, dose of 0.6-0.8 IU/mL vs. placebo	Dalteparin 100 IU/kg, SC, 2x/daily, vs. aspirin 160 mg, daily	Tinzaparin 175 IU/kg or 100 IU/kg, SC, daily vs. aspirin 300 mg/day	Certoparin 3,000 IU (aXa), SC, daily or 2x/daily, 5,000 IU (aXa), 2x/daily, or 8,000 IU (aXa), 2x/daily	Nadroparin calcium 3,800 IU, SC, 2x/daily, vs. aspirin 160 mg/day	Argatroban (100 µg/kg), IV, followed by continuous infusion of 3 µg/kg/min or 1 µg/kg/min vs. placebo
Duration of anticoagulation	10 days*	14 days*	7 days	14 days	10 days*	12-16 days	10 days*	5 days
Time between onset of symptoms and anticoagulation	Within 48 hours	Within 48 hours	Within 24 hours	Within 30 hours	Within 48 hours	Within 12 hours	Within 48 hours	Within 12 hours
Number of patients involved	308	19,435	1,281	449	1,486	404	603	13,342
Short-term outcomes	No differences at functional or bleeding rates between the groups at 10 days	↓ Ischemic stroke and pulmonary embolism; ↑ Hemorrhagic stroke and extracranial bleeding at 14 days	In general, ↑ bleeding. More favorable outcomes among large-artery atherosclerosis patients at 7 days	↑ Symptomatic intracranial hemorrhaging; Dalteparin showed no benefit in prevention of recurrent stroke at 14 days	↑ Intracranial hemorrhaging, particularly at high doses within 24 hours; ↓ Venous thrombotic events with high dose tinzaparin	No functional difference between groups. Clinical improvement in all groups within 14 days. Severe bleeding more frequent in highest doses	Hemorrhagic transformation and adverse events were similar across groups	Bleeding rates were similar among groups; no major bleeding; neurological scores did not differ among the groups
Long-term outcomes	Significant dose-dependent reduction in risk of death or dependency at 6 months with LMWH	Heparin showed no benefit for disability or death at 6 months ↑ Rate of patients with complete regression of symptoms using aspirin	More favorable outcomes among large-artery atherosclerosis patients at 3 months	No differences between groups for independence or death at 6 months	No differences between groups for independence at 3 or 6 months	Treatment groups were not different with respect to favorable functional outcome at 90 days	Patients with Barthel Index ≥ 85% at 6 months: 73% in use of nadroparin and 69% using aspirin	Neurological scores did not differ among the groups at 90 days

IST: International Stroke Trial; TOAST: Trial of ORG 10172 in Acute Stroke Treatment; HAEST: Heparin in Acute Embolic Stroke Trial; TAIST: Tinzaparin in Acute Ischaemic Stroke Trial; FISS-tris: Fraxiparin in Stroke Study for the treatment of ischemic stroke; IU: international unit; LMWH: low-molecular-weight heparin; UFH: unfractionated heparin; IV: intravenous; SC: subcutaneous; 2x/daily: twice daily; aXa: anti-factor X.
*Patients remained on secondary prevention of stroke using antiplatelet therapy after anticoagulation.

Discussion

Only two out of eight studies favored the early anticoagulation for acute stroke. These studies presented different sample selection, anticoagulation agents, outcomes, time of follow-up, and clinical measures. Most studies have used LMWH as the anticoagulant agent and two of them used unfractioned heparin and a particular direct thrombin inhibitor, i.e., argatroban.[23-25] Thus, it is very difficult to include the whole data in a meta-analysis. Three meta--analyses were done regarding the use of anticoagulants for acute ischemic stroke between 2002 and 2008 with different inclusion criteria and studies that were included.[26-28] Thereafter, it is necessary to evaluate each study individually to permit a critical analysis of the results.

The study conducted by Kay et al. was the first clinical trial to evaluate the use of LMWH nadroparin for the treatment of 308 subjects with acute stroke. Death and dependency were the main outcomes. A significant, dose-dependent reduction in the risk of death or dependency was observed after 6 months among the subjects treated with nadroparin. Bleeding rates were similar among the groups.[12]

After two years, the results of the International Stroke Trial (IST) comparing the administration of subcutaneous unfractionated heparin (UFH) versus aspirin within 48 hours of onset of ischemic stroke symptoms were published. This randomized trial involved 19,435 patients from 467 hospitals in 36 different countries. The study showed that patients treated with heparin had a significantly lower recurrence of ischemic stroke within a 14-day period. However, there was a significant increase in the risk of hemorrhagic stroke and extracranial bleeding in this group. Over a 6-month follow-up, anticoagulation offered no benefits in terms of rates of functional decline or death.[13]

In the following year, results were published for the Acute Stroke Treatment (TOAST), assessing the use of a bolus of intravenous danaparoid, followed by continuous infusion for 7 days, within 24 hours of stroke symptom onset. The trial assessed 1,281 patients and found that early administration was associated with a greater likelihood of bleeding. Ischemic strokes were classified by subtype into the following categories: large-artery atherosclerosis, cardioembolism, small-artery occlusion and other determined or undetermined causes. A significant response to the treatment was observed at both 7 days and 3 months among individuals with stroke secondary to large-artery atherosclerosis.[14]

In the HAEST, anticoagulation with dalteparin was initiated within 30 hours of symptom onset in patients with ischemic stroke and atrial fibrillation. The primary objective was to determine whether the anticoagulant reduced stroke recurrence in 14 days. In all, 449 patients were included and no benefit in the prevention of recurrent stroke compared to aspirin was evident. In addition, patients using anticoagulants had more symptomatic brain hemorrhages. No difference in functional outcomes or deaths was observed at 14 days or at 6 months.[15]

In the TAIST trial, administration of a moderate-to--high dose of tinzaparin was compared to aspirin. In all, 1,484 patients from 100 centers located in Europe and Canada were involved, all treated within 48 hours of symptoms onset. High-dose tinzaparin was associated with lower occurrence of venous thromboembolic events. However, the rate of symptomatic intracranial hemorrhage was significantly higher at the higher doses of the anticoagulant, more frequently when the treatment was started within 24 hours of ischemic stroke. The Rankin scale and Barthel index, measuring the degree of disability acquired by the patient after stroke in their daily living activities, revealed no differences at 3 or 6 months between tinzaparin and aspirin. Given the absence of functional improvement in outcomes and the greater risk of bleeding, high doses of LMWH were not recommended as routine treatment. Possible scaling of treatment was discussed, with initial prescription of aspirin at the time of diagnosis followed by low-dose LMWH as prophylaxis for venous thromboembolism after 1 or 2 days, when the risk of intracranial hemorrhaging has declined.[16]

The TOPAS trial randomized 404 subjects within 12 hours of an acute stroke onset to four different treatment regimens with LMWH certoparin. After three months of follow-up, no significant difference was observed in functional outcome (Barthel index) or neurological recovery (European Stroke Scale). Severe bleeding complications were more frequently observed in the highest-dose group.[17]

Nadroparin calcium was compared with aspirin in the FISS-tris trial, which involved 603 Asian patients with large-artery occlusive disease. The therapy was initiated within 48 hours of onset of symptoms and patients were followed up until six months after the ischemic stroke and assessed by the Barthel index and Rankin scale. The results showed no significant benefit of nadroparin over aspirin in the patients assessed, and further investigation of anticoagulation in large-artery atherosclerosis patients was recommended.[18]

The evidence of benefits from anticoagulation after acute stroke due to large-artery arteriosclerosis is also controversial. As previously outlined, in the TOAST trial a significant response to danaparoid treatment was observed at both 7 days and 3 months among individuals with ischemic stroke secondary to large-artery atherosclerosis.[14]

However, for treatment to be effective, diagnosis of this stroke subtype must be early and accurate, requiring ancillary exams such as carotid and transcranial Doppler, angioresonance and angiotomography.[14] The FISS-tris trial recommends further investigation of anticoagulants in large-artery atherosclerosis, particularly intracranial.[18]

Argatroban is a direct thrombin inhibitor that, unlike heparin, can bind to thrombin in circulation or adhered to the clot, and does not greatly prolong activated partial thromboplastin time at various doses. In addition, it does not induce the formation of antibodies or interact with heparin-induced antibodies.[23,24] Consequently, the agent causes less bleeding, does not induce or potentialize heparin-induced thrombocytopenia, and is well-tolerated.[23] Its use has proved relatively safe in cases of ischemic stroke and heparin-induced thrombocytopenia and is available in Japan for the treatment of stroke due to large-artery atherosclerosis.[25] Argatroban is short-acting (half-life of 39-51 minutes) and can be monitored by tests such as prothrombin time and activated partial thromboplastin time.[23]

Argatroban anticoagulation in patients with acute ischemic stroke (ARGIS-1) was a randomized trial in an effective population of 13,342 patients from North American centers with up to 12 hours since onset of ischemic stroke symptoms and a National Institute of Health Stroke Scale (NIHSS) of 5 to 22. Patients were divided into three subgroups (high argatroban infusion; low argatroban infusion and placebo) undergoing treatment for five days. An initial intravenous bolus of argatroban (100 μg/kg) was administered, followed by continuous infusion of 3 μg/kg/min (high dose) or 1 μg/kg/min (low dose). Patients received standard care for stroke, including aspirin (81-325 mg) in the first 48 hours of symptom onset. With regard to intracranial hemorrhage, no differences were found among the groups; no major bleeding occurred; minor systemic hemorrhage increased only at high argatroban dose relative to placebo. Neurological scores did not differ among the groups at hospital discharge or at 90 days. Thus, it was concluded that argatroban infusion within 12 hours of symptoms at an average dose of 1.2 to 2.7 μg/kg/min for five days promoted relatively safe anticoagulation in cases of ischemic stroke. Given that argatroban has proven safety, other studies further assessing its efficacy should be performed.[19]

Finally, the utility and safety of the use of NOAC agents for acute cardio-embolic stroke within 7 to 14 days after onset remain unclear according to the best of our evidence. NOAC agents present a more rapid effect and less hemorrhagic complications compared with warfarin, and appear to provide effective anticoagulation for acute cardio-embolic stroke.[29] Future studies like the Triple AXEL can better evaluate the efficacy of NOAC agents in this scenario. The Triple AXEL trial uses rivaroxaban for early anticoagulation of ischemic stroke.[30]

As we focus our attention on anticoagulation treatment after acute ischemic stroke, several particular clinical scenarios were kept out of this review such as cerebral venous thrombosis, arterial dissection, thromboembolism following acute stroke. These topics were not included in our review.

Conclusion

There are numerous controversies over anticoagulation in the management of ischemic stroke. The ideal timing for initiating anticoagulation remains undefined, requiring further investigation. In general, early anticoagulation for ischemic stroke is not recommended, with few exceptions, as might be the case with argatroban, a drug that can bind to thrombin in circulation or adhered to clot and does not prolong activated thromboplastin time at various doses, and used for the treatment of strokes secondary to large artery atherosclerosis. Further studies are still needed to confirm drug efficacy and possible side effects. Other exceptions may be coagulopathies such as idiopathic and familial thrombophilia and acute myocardial infarction with formation of mural thrombus, but the need for weight pros and cons is still warranted. Furthermore, the development of antidotes for these anticoagulants and establishing a means of measuring their therapeutic effect can allow safer clinical practice.[31-33]

Further large-scale trials correlating treatment with factors potentially associated with greater risk of thrombotic events (history of venous thromboembolism, thrombophilia or cancer) or hemorrhagic events (minor cerebral hemorrhages and more in-depth analysis of neuroimaging) are obviously needed, especially considering the escalating incidence of cerebrovascular complications in an aging population and its severe and several sequelae.[34,35]

Therapeutic decisions could be more safely guided by some important factors, such as the volume of acute lesions, etiology, age, gender, and time of occurrence (i.e., how many hours after the ictus is the anticoagulation safe). Future studies correlating this information with anticoagulation in the acute phase could help improve the safety of this practice.[36,37]

Resumo

Anticoagulação no acidente vascular cerebral: uma revisão sistemática

Introdução: O acidente vascular cerebral (AVC) é uma das doenças mais importantes no mundo. Vários cenários clínicos exigem dose completa de anticoagulantes para tratar a etiologia primária do AVC ou para o tratamento de uma comorbidade. Contudo, existem inúmeras controvérsias em relação ao tratamento com anticoagulação no AVC, como tempo para o início, eficácia de acordo com a etiologia do AVC, dose ideal de anticoagulante e utilização de novos anticoagulantes.
Método: Busca computadorizada de ensaios clínicos controlados e randomizados foi feita até a presente data nas bases Medline, Scielo, Embase, PsychInfo e Cochrane Library, usando termos MeSH e as palavras-chave acidente vascular cerebral, acidente vascular cerebral isquêmico, anticoagulação, anticoagulantes, heparina de baixo peso molecular, heparina, varfarina, dabigatran, rivaroxaban, apixaban. O modelo PRISMA foi utilizado para avaliar os ensaios clínicos.
Resultados: Catorze ensaios clínicos foram selecionados de acordo com critérios de inclusão. Não foram encontradas evidências que apoiam o uso precoce de heparina, heparinoides ou heparina de baixo peso molecular (HBPM) precocemente após o AVC. Nenhuma evidência consistente para o uso de warfarina e anticoagulantes orais mais recentes foi encontrada. Argatroban foi o único anticoagulante com resultados positivos significativos para AVC isquêmico precoce não embólico.
Conclusão: O momento ideal para iniciar a anticoagulação continua mal definido, exigindo uma investigação mais aprofundada. Anticoagulação precoce para AVC isquêmico não é recomendada, com exceção para o argatroban.

Palavras-chave: acidente vascular cerebral isquêmico, anticoagulação, heparina, varfarina.

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Decompensated chagasic heart failure versus non-chagasic heart failure at a tertiary care hospital: Clinical characteristics and outcomes

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Summary

Objective: To evaluate clinical and epidemiological characteristics and clinical outcomes in patients hospitalized with decompensated heart failure (DHF), with a comparison between Chagas and non-Chagas disease.
Method: This is a retrospective cohort study involving 136 patients consecutively admitted with DHF between January 1 and December 31, 2011, with the following outcomes: acute renal failure, cardiogenic shock, rehospitalization, and hospital death. Individuals aged ≥ 18 years with DHF were included while those with more than 10% of missing data regarding outcomes were excluded. Statistical analysis was performed using SPSS version 17.0. Chi-squared test was used to compare proportions. Student's T test was used to compare means. Kaplan-Meier and log-rank tests were used to compare rehospitalization rates between the two groups over time.
Results: Chagasic and non-chagasic patients were compared. The first had lower mean systolic blood pressure (111.8±18.4 *versus* 128.8±24.4, p<0.01), lower mean diastolic blood pressure (74.5±13.6 *versus* 82.0±15.2, p<0.01) and lower left ventricular ejection fraction (26.5±6.2 *versus* 41.5±18.9, p<0.01). In all, 20 patients with Chagas (50.1%) were rehospitalized, compared to 35 patients in the non-Chagas group (35.4%, p=0.04). Log rank test = 4.5 (p<0.01) showed that rehospitalization rates between the two groups over time (Kaplan-Meier curves) differed.
Conclusion: Chagas disease was associated with lower systolic and diastolic blood pressure and lower left ventricular ejection fraction. The rehospitalization rate was higher in Chagas disease.

Keywords: heart failure, chagas cardiomyopathy, patient readmission.

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Introduction

Heart failure (HF) is an important public health problem, affecting around 23 million people worldwide.[1] It is a pathological condition, with high mortality, that can exceed 50% within five years after diagnosis.[2]

The age group older than 60 years is the most affected by the syndrome, with more than 70% of hospitalizations occurring at this stage of life. National forecasts indicate that in 2025 Brazil will have the sixth largest elderly population, with approximately 30 million people, which should result in the multiplication of cases of HF and spending on the syndrome.[3]

The clinical presentation generally has an impact on quality of life, causing disability and dependency. In some

cases, its etiology is difficult to establish due to the presence of multiple risk factors and irregular lifestyle habits.[4]

Among the possible etiologies of HF, Chagas disease (CD) has been showed in some studies as that with the worst prognosis.[5] The state of Bahia still presents endemic outbreaks of CD and significant mortality related to its complications. Changes in the disease presentation patterns, the influence of treatment progression and outcomes need to be demonstrated, given that the largest registries of heart failure in the world usually do not include CD, which is a rare disease in most developed countries. Thus, studies are needed in populations where CD is endemic, such as in Bahia. The aim of this study was to assess the clinical characteristics and intrahospital outcomes, seeking to compare such aspects in chagasic versus non-chagasic patients.

Method

Study design

This is a retrospective cohort study with data collected from the time of hospitalization up to the patient's discharge from hospital or death.

Population

The reference population for the study corresponded to adult individuals who were admitted with ICD I500 in the period from January 1 to December 31, 2011. The patients originated from clinical wards, intensive care units, the emergency room or those transferred from other units within the hospital.

The hospital where the data was collected (Hospital Santo Antônio) is located in the city of Salvador, BA, Brazil. This is a tertiary care hospital with 1,090 beds, and only serves patients via the public health system.

Sampling

The list of patients hospitalized for decompensated heart failure (DHF) in the period from January 1, 2011 to December 31, 2011 was obtained through a report provided by the archive service at the hospital where the study was conducted.

Constitution of the sample initially occurred upon request of the medical records from the service, with a total of 265 hospitalizations. The medical records of the first 136 hospitalizations were eligible. We excluded 129 hospitalizations whose data was scarce or those that related to subsequent hospitalizations in the period studied.

Inclusion criteria

Patients hospitalized due to DHF (ICD I500) aged 18 years or older. In the case of hospital readmission, only the first hospitalization within the period was considered.

Exclusion criteria

Insufficient medical record data with more than 10% of data missing in relation to the outcomes of the study.

Operationalization of data collection

Forty-one variables were collected using a standardized instrument for each patient included in the study. The data was obtained from hard copy medical records and computerized records of laboratory tests.

Independent variables: age, sex, ethnicity, marital status, occupation, cost of hospitalization, family history of heart disease, blood pressure, heart rate, respiratory rate, comorbidities such as: hypertension, diabetes, dyslipidemia, obesity, alcoholism, history of stroke, use of a pacemaker, anemia upon admission, chronic renal failure, edema, dyspnea, precordialgia, jugular stasis, hepatomegaly, and etiology of the HF.

Dependent variables: percentage of rehospitalizations, acute kidney damage defined as increased serum creatinine levels greater than 50% compared to the baseline creatinine, cardiogenic shock as mentioned in the medical records, and in-hospital death.

Data on rehospitalizations were initially collected using internal hospital records and later confirmed via telephone contact with the patient or family, considering all the rehospitalizations of the patients in the hospital during the period studied.

Statistical analysis

We used Statistical Package for Social Sciences (SPSS) version 17.0 for the statistical analysis.

The qualitative variables were expressed as absolute values and valid percentages. The quantitative variables were expressed as means ± standard deviation in the case of a Gaussian distribution.

Descriptive statistics was used for determination of the frequencies, measures of central tendency and dispersion of the variables of interest. We used a Chi-squared test for comparison between proportions. Student's T-test was used to compare averages in the case of Gaussian distribution of the variable.

We used Kaplan-Meier curves for comparison of the rehospitalization rates in the Chagas disease group versus the non-Chagas group over time. Log-rank test = 4.5 ($p<0.01$).

Alpha-error of 0.05 was adopted for all of the statistical analyses.

Results

Between January 1 and December 31, 2011, there were 265 hospitalizations due to HF at Hospital Santo Antônio. A

total of 129 patients were excluded, leaving 136 patients for analysis. The most frequently described etiology was hypertensive cardiomyopathy, affecting 42% of cases, followed by 26% Chagas cardiomyopathy, ischemic heart disease (18%), valvular heart disease (3%), and unknown causes (11%).

The average age was 59±13 years, ranging from 23 to 89 years, and 71.3% were male. In relation to ethnicity, there was a predominance of those of African descent, with a frequency of 83.8%. The sociodemographic characteristics are shown in the Table 1.

TABLE 1 Sociodemographic characteristics of patients admitted to hospital with decompensated heart failure. Salvador, 2011 (N=136).

Variable	Frequency	
Gender	Male	71.32% (97)
	Female	28.67% (39)
Age	59.71±13.86	
Ethnicity	African-descended	83.82% (114)
	Non-african-descended	8.08% (11)
Occupation	Actively working	34.55% (47)
	Retired	50 (49.50%)
	Homemaker	16 (15.84%)
	Unemployed	27 (26.74%)
Marital status	Common-law partner	31.61% (43)
	Widow(er)	14.70% (20)
	Single	35.29% (48)
	Divorced	7.35% (10)
Drinking habit	46 (33.82%)	
Smoking habit	31 (22.79%)	
Arterial high blood pressure	88 (64.70%)	
Diabetes mellitus	32 (23.52%)	
Dyslipidemia	8 (5.88%)	
Obesity	9 (6.61%)	
Anemia	66 (48.52%)	
History of stroke	14 (10.29%)	
Use of pacemaker	9 (6.61%)	
SBP	124±24	
DBP	79±15	
HR	82±17	
RR	24±7	
Edema	121 (88.97%)	
Dyspnea	127 (93.38%)	
Functional class	I – 02 (1.47%)	
	II – 11 (8.08%)	
	III – 30(22.05%)	
	IV – 86(63.23%)	

Quantitative variables are expressed as mean ± standard deviation. Qualitative variables are expressed as a valid percentage (absolute N). SBP: systolic blood pressure; DBP: diastolic blood pressure; HR: heart rate; RR: respiratory rate.

In relation to the clinical characteristics upon admission, there was a predominance of patients with functional class IV (86 patients, 63.2%), demonstrating impairment in the completion of daily activities, with nocturnal paroxysmal dyspnea and dyspnea on minimal exertion. The most common comorbidities were hypertension, anemia, diabetes, dyslipidemia, and obesity.

The clinical characteristics upon admission, comparing patients with Chagasic etiology with those of other etiologies, are shown in Table 2.

Among the possible outcomes considered, there were 22 deaths and 55 rehospitalizations. Acute renal damage affected 88 patients. The evaluation of the outcomes shows that chagasic patients were rehospitalized more than non-chagasic patients; p=0.04 (Table 3). The comparison of the Kaplan-Meier curves between the two groups over time showed that: all rehospitalizations of Chagasic heart disease patients occurred in 100 to 200 days, while rehospitalization among the non-chagasic patients occurred in 300 to 365 days with a log rank test = 4.5; p<0.01 (Figure 1).

Discussion

Despite scientific evidence showing a reduction of its prevalence in Brazil, Chagas disease constitutes a major public health problem and an important cause of heart failure in certain regions of the country.[6] In our sample, we found that among the etiologies of HF, the most frequent was hypertensive heart disease, followed by chagasic heart disease.

Other studies that included patients with DHF have found different etiological distributions. In a serological survey on the prevalence of human Chagas disease conducted in Brazil, the states of Minas Gerais, Rio Grande do Sul, and Bahia presented the highest prevalence.[6,7]

Braga et al.[8] conducted a study in Salvador with patients accompanied at outpatient clinics, showing that Chagasic heart disease was the most frequent etiology, totaling 48% of cases. Although located in the same city, our sample presented a different etiological distribution, possibly due to a time difference between the surveys, given the progressive reduction in the prevalence of Chagas disease. Freitas et al. monitored outpatients and found that chagasic patients showed a relative risk 2.26 to 2.97 higher in relation to mortality compared to other heart failure etiologies.[9]

In our study, the average age of chagasic patients was not significantly lower than that of non-chagasic patients. Some studies have shown an average age that is even lower in the chagasic group as a result of the pathogen-

TABLE 2 Clinical and therapeutic characteristics of patients according to the main etiology of heart failure (N=136).

Variables	Chagas (n=37)	Non-Chagas (n=99)	p-value
Age	57.6±11.4	60.5±14.7	0.27
Male gender	29 (78.4%)	68 (68.7%)	0.26
Functional class I	1 (2.70%)	1 (1.01%)	0.86
Functional class II	3 (8.10%)	8 (8.08%)	
Functional class III	8 (21.62%)	22 (22.22%)	
Functional class IV	23 (62.16%)	63 (63.63%)	
Systolic BP (mmHg)	111.8±18.4	128.8±24.4	<0.01
Diastolic BP (mmHg)	74.5±13.6	82.0±15.2	0.01
Heart rate (bpm)	75.7±15.7	84.7±17.9	0.01
Edema	35 (94.6%)	85 (85.9%)	0.67
Carvedilol in mg/day	12.70±4.97	13.03±5.62	0.77
Captopril in mg/day	93.42±15.02	97.70±15.68	0.51
Furosemide in mg/day	61.05±20.24	63.47±19.48	0.55
Dobutamine (µg/min)	350±350	418.33±229.47	0.73
Jugular engorgement	18 (48.6%)	37 (37.4%)	0.13
Anemia	18 (48.6%)	48 (48.5%)	0.98
Ejection fraction (EF)	29.5±6.2	41.5±18.9	0.01
History of stroke	2 (5.4%)	12 (12.1%)	0.25
Use of pacemaker	5 (13.5%)	4 (4.0%)	0.04

BP: blood pressure. (*) The quantitative variables are expressed as means ± standard deviation, while the qualitative variables are expressed as absolute values (valid percentage).

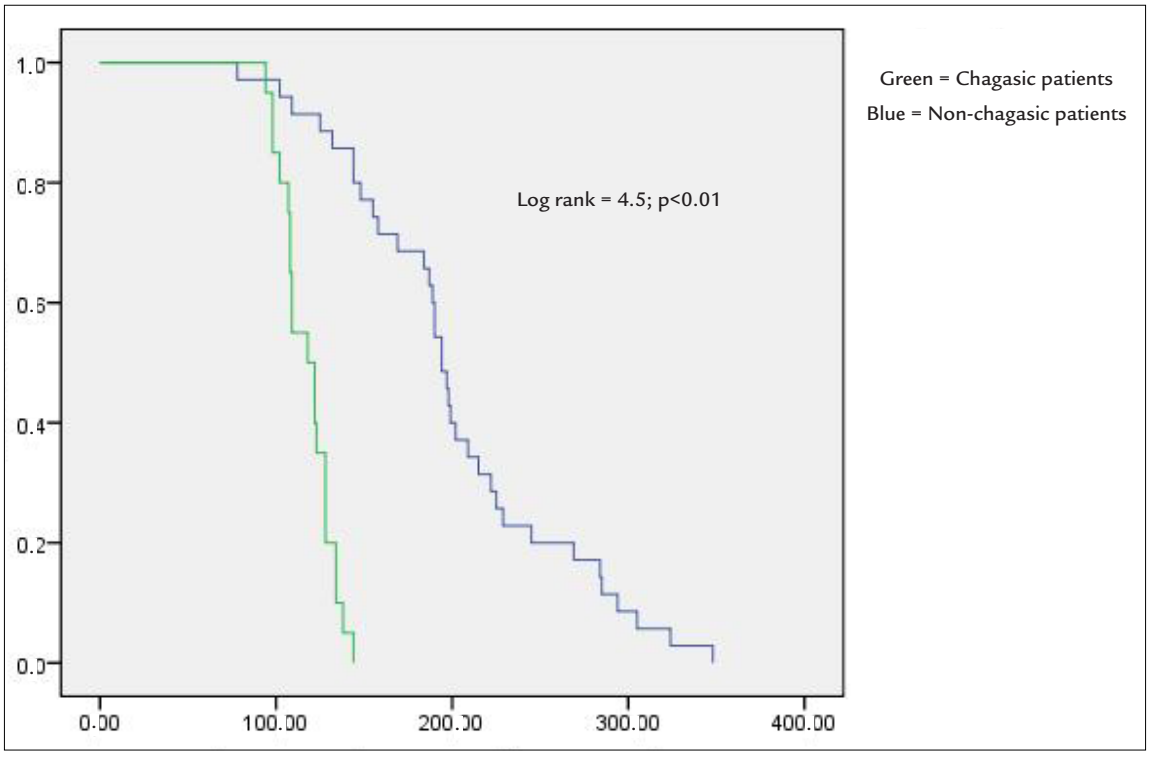

FIGURE 1 Kaplan-Meier curves showing different intervals in days for the occurrence of rehospitalization between chagasic patients and non-chagasic patients.

TABLE 3 Type of clinical outcomes and their correlation with chagasic or non-chagasic heart failure etiology (N=136).

Outcomes	Chagas (n=37)	Non-Chagas (n=99)	p-value
Hospital death	18.91% (7)	15.15% (15)	0.59
Rehospitalization	54.05% (20)	35.35% (35)	0.04
Acute kidney damage	62.16% (23)	68.42% (65)	0.49
Cardiogenic shock	8.10% (3)	6.31% (6)	0.71
Arrhythmia	24.32% (9)	14.73% (14)	0.19

esis of the disease, such as the study by Cardoso et al.,[10] which found an average age of 53 years.

In relation to gender, we identified that 71% of the patients with DHF in general were male, and 78% of HF patients with chagasic etiology were male, as corroborated by other studies[11-13] assessing elderly patients hospitalized due to the disease in our country. Braga et al.,[8] on the other hand, identified the male sex in 49% of the sample of chagasic patients and 55% in non-chagasic patients.

When comparing the clinical characteristics of the chagasic and non-chagasic populations there was significant difference for certain variables. Cardoso et al.[10] conducted a cohort study that assessed patients with clinical hemodynamic profile C, according to the classification given by Stevenson. Systolic blood pressure (SBP) was compared in chagasic and non-chagasic patients, resulting in 89.3±17.1 mmHg versus 98.8±21.7 mmHg (p=0.03). In our study, we did not select patients based on their hemodynamic profile, finding an average SBP of 111.8±18.40 in chagasic patients versus 128.8±24.40 in non-chagasic patients (p<0.01). Braga et al.[8] also identified lower SBP in chagasic patients, with 121±22 mmHg compared to non-chagasic patients, with 130±26 mmHg (p=0.001). Diastolic blood pressure was also significantly lower. These findings may be justified by the fact that chagasic patients exhibit more extensive myocardial destruction compared to other etiologies.[12]

Heart rate was found to be lower in chagasic patients, with 75.7±15.7 versus 84.7±17.9, with the same occurring in the study by Braga[8] and Nogueira.[7] Silva et al.[12] relate this fact to sinus dysfunction and alteration of the autonomic nervous system, which is more common in this group.

We identified significantly lower ejection fraction in the chagasic group, which is justified by the physiopathology of CD with cardiac involvement due to extensive myocardial destruction, tissue perfusion disorders associated with dysautonomia and the presence of cardiac arrhythmias. Silva et al. also identified similar findings.[12] Melo et al. studied the profile of prothrombotic and pro-inflammatory markers between chagasic and non-chagasic patients in a cross-sectional cohort study, concluding that both groups had exacerbated inflammatory activity. However, the prothrombotic status was more prominent among non-chagasic patients.[14]

Considering all outcomes analyzed, frequency of rehospitalizations during the period studied was the only isolated outcome presenting a significant difference while comparing chagasic and non-chagasic patients (54.1% versus 35.4%), respectively (p=0.04). Higher frequency of rehospitalization is a marker of poor prognosis and translates into greater morbidity among chagasic patients compared to non-chagasic patients.

Recent scientific evidence has indicated a protective factor related to the use of beta blockers. Issa et al. demonstrated that the use of beta blockers has a beneficial effect on the survival of patients with heart failure caused by Chagasic diseases compared to other etiologies.[15] Ayub-Ferreira et al. showed that Chagas disease was an independent predictor of death due to heart failure and all causes of death. These authors also found reduced mortality from cerebrovascular accident, other non-cardiac causes of death and longer hospital stay while comparing chagasic and non-chagasic heart diseases patients.[16] In more severe cases of Chagas disease, the only treatment capable of modifying the natural history of disease is a heart transplant. Fiorelli et al. conducted a review of 107 transplant patients with a focus on disease reactivation, rejection, and mortality. The authors found the trypanosome in the myocardium of 71.8% of the transplant patients, in addition to other tissues. Hospital mortality was 17.7% due to infection, graft dysfunction, or sudden death. Late mortality was 25.2% due to rejection, infection, lymphoma, sarcoma, constrictive pericarditis, and reactivation of Chagas disease in the central nervous system.[17]

Clinical, socioeconomic, and organizational risk factors related to healthcare providers also have an influence.

Monitoring should be carried out by a multidisciplinary team composed of nurses, doctors, psychologists, social workers, nutritionists, and pharmacists. A randomized study identified benefits regarding knowledge of the disease and self-care in patients exposed to an educational nursing intervention.[18]

Limitations

Some of the limitations of the present study are related to its clinical design. This is a retrospective study, involving observation with information retrieved from medical records gathered in several folders. The quality of the data in the medical records and the absence of systematization of the records may impair the accuracy of data collection. In addition, some patients were excluded because no serology was found to confirm or rule out the diagnosis of Chagas disease.

Another important limitation is the small sample size, which could prevent the identification of differences in low-incidence outcomes.

Conclusion

Chagas disease patients presented more severe clinical profiles than patients with other HF etiologies, with lower left ventricle ejection fraction, lower systolic blood pressure, lower diastolic blood pressure, and reduced heart rate, as well as a higher percentage of rehospitalizations.

More effective public health actions to prevent and control vector-borne transmission and transfusion transmission are necessary in our country, which still has a significant prevalence of Chagas disease.

Resumo

Insuficiência cardíaca descompensada chagásica *versus* não chagásica em um hospital de atenção terciária: características e desfechos clínicos

Objetivo: Avaliar características clínico-epidemiológicas e desfechos clínicos em pacientes internados por insuficiência cardíaca descompensada (ICD), estabelecendo uma comparação entre pacientes chagásicos e não chagásicos.

Método: Trata-se de um estudo de coorte retrospectivo abrangendo 136 pacientes internados consecutivamente com ICD entre 1 de janeiro e 31 de dezembro de 2011, tendo como desfechos: lesão renal aguda, choque cardiogênico, reinternamento e óbito hospitalar. Foram incluídos indivíduos com idade ≥ 18 anos com ICD e excluídos aqueles com mais de 10% de dados faltantes em relação aos desfechos. Para a análise estatística, foi utilizado o SPSS® versão 17.0. Para a comparação entre proporções, foi utilizado o teste Qui-quadrado. O teste T de Student foi utilizado para comparar médias. Utilizamos as curvas de Kaplan-Meier e o teste do *log rank* para comparar as taxas de reinternações entre os dois grupos ao longo do tempo.

Resultados: Na comparação entre chagásicos e não chagásicos, os primeiros apresentaram menor média de pressão arterial sistêmica (111,8±18,4 *versus* 128,8±24,4; p<0,01), menor média de pressão arterial diastólica (74,5±13,6 *versus* 82,0±15,2; p<0,01) e menor fração de ejeção do ventrículo esquerdo (26,5±6,2 *versus* 41,5±18,9; p<0,01). Um total de 20 chagásicos (50,1%) reinternaram contra 35 não chagásicos (35,4%; p=0,04). O teste do *log rank* = 4,5 (p<0,01) mostrou que as taxas de reinternações entre os dois grupos ao longo do tempo (curvas de Kaplan-Meier) diferiram.

Conclusão: A doença de Chagas associou-se a menores valores de pressão arterial sistólica e diastólica, além de menor fração de ejeção do ventrículo esquerdo. A taxa de reinternamento foi maior em chagásicos.

Palavras-chave: insuficiência cardíaca, miocardiopatia chagásica, readmissão do paciente.

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Clinical hypoxic-ischemic encephalopathy score of the Iberoamerican Society of Neonatology (Siben): A new proposal for diagnosis and management

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Summary

Hypoxic ischemic encephalopathy is a major complication of perinatal asphyxia, with high morbidity, mortality and neurologic sequelae as cerebral palsy, mostly in poor or developing countries. The difficulty in the diagnosis and management of newborns in these countries is astonishing, thus resulting in unreliable data on this pathology and bad outcomes regarding mortality and incidence of neurologic sequelae. The objective of this article is to present a new clinical diagnostic score to be started in the delivery room and to guide the therapeutic approach, in order to improve these results.

Keywords: asphyxia, hypoxic ischemic encephalopathy, Siben Neurological Score.

Introduction

Hypoxic ischemic encephalopathy (HIE) is a disease with a strong potential to cause cerebral palsy. Depending on its severity, it may turn into an emotional, social, and economic tragedy for the individual and their entire family, as well as society as a whole.

The pathophysiology of HIE has been described in detail, with a primary phase leading to neuronal apoptosis[1] and a secondary reperfusion phase that occurs about six hours after the hypoxic ischemic insult, with various types of response that may lead to a secondary neuronal injury, compatible with the presence of neurologic sequelae in the newborn.

HIE is characterized by neurological symptoms of variable severity, and is classified as mild, moderate and severe. The condition is accompanied by the involvement of one or more organs and may lead to multiple organ failure. The symptoms vary according to the severity of the disease and whether there is central nervous system involvement, ranging from deterioration of wakefulness up to seizures and coma; or from a multi-organ point of view, involvement of the digestive tract (from vomiting up to symptoms of necrotizing enterocolitis), the urinary tract (from oliguria up to acute renal failure[5]), the respiratory system (pulmonary hemorrhage up to pulmonary hypertension[2]) and the cardiovascular system, often with sinus bradycardia[3] (predominance of the parasympathetic system), and, finally, metabolic disorders (calcium, glucose and magnesium[4]). All of these changes may aggravate the involvement of the central nervous system.

Currently, perinatal asphyxia associated with moderate or severe HIE, which is its main complication, affects between 1-2/1,000 live births in developed countries and is estimated at affecting between 10-20/1,000 live births in poor or developing countries,[6] being responsible for 1/3 of neonatal mortality in these countries. However, we believe that the actual incidence of HIE in poor and/or developing countries is unknown. What we do know is that in Brazil 13 to 15 newborns die every day as a result of asphyxia, according to a study by the resuscitation committee of the Brazilian Pediatric Society.[7] There are several factors making us unaware of our HIE rate, as published by Perez.[8] These include the difficulty presented by health teams to assess the newborns neurologically, or a belief that an Apgar score of < 3 or 4 at 5' and/or 10' is sufficient and necessary to rule out the diagnosis of HIE. A total lack of preparedness among health teams in relation to the use of neurological scores for the newborn or

the total lack of their use at some centers is prevalent. These are some of the factors leading us to believe that the actual incidence of HIE is higher than that already published. Having an easy-to-use clinical evaluation mechanism from the moment of birth would favor the obtainment of a more realistic notion of the importance of this pathology in Brazil and in all developing countries, as well as a better therapeutic outcome for such patients.

Objective

To present a simple clinical neurological score for hypoxic ischemic encephalopathy developed by the Iberoamerican Society of Neonatology (Siben) and a flow chart to guide the diagnosis and management of newborns who have suffered some degree of asphyxia.

Diagnosis of HIE and description of the Siben HIE score

Considering a maternal obstetric history with the identification of hypoxic ischemic insult (e.g. umbilical cord prolapse, uterine rupture) associated with the presence of a low Apgar score and umbilical cord pH with acidosis ≤ 7.1 leads us to a diagnosis of HIE. However, proper neurological clinical examination of the newborn is essential to confirm the diagnosis.

In order to systematize this neurological assessment of newborns, several neurological scores have been created for newborns, such as the currently available Sarnat and Sarnat,[9] Thompson,[10] and Garcia-Alix[11] scores, as well as others. None of these have been applied since the first minutes of life, and some of them are complex and/or use supplementary exams.

In our view, a neurological score, including only clinical aspects that can be started as soon as we finish the Apgar score, is essential. Therefore, we have developed the neurological score presented in Table 1.

This scale assesses ten clinical aspects and is very simple to perform. As HIE has been previously classified as mild, moderate or severe, each item evaluated varies according to the degree of severity. For example, if spontaneous activity is absent, the corresponding item is found in severe HIE (Table 1). A point is given to every item that corresponds to a level in the Siben score, with the diagnosis of HIE considered as strongly suspect at a certain level, in the presence of three points or more. In the event of finding items at different levels, the level with the most items found will predominate. For example, if a slight change in the level of consciousness, such as being hyperalert, and a change in posture with mild distal flexion and weak suction are found in a newborn with a history of a hypoxic ischemic insult, we could classify this newborn as presenting mild HIE. However, if convulsions, bradycardia, hypotonia, and a weak Moro reflex are presented in association, we have a case in which signs of moderate HIE predominate over the mild condition, and this should be the established diagnosis. In short, the mild, moderate or severe HIE diagnosis will predominate according to the highest number of items (always above three) found in the corresponding level.

The neurological score that we have developed is shown in Table 1, and is called the HIE Score of the Iberoamerican Society of Neonatology.

We use the Siben Neurological Score for all infants who have an Apgar ≤ 5 in the first, fifth or tenth minute, and we begin using it in the delivery room as soon as the Apgar score is concluded. A diagnosis made as early as possible will guide the treatment and possibly lead to a more favorable outcome in the clinical case.

Unlike other neurological scores, our tool only relies on clinical criteria for the score, which greatly facilitates clinical monitoring and, in our opinion, the early diagnosis or early suspected diagnosis of HIE. In many centers with high birth rates, prompt evaluation by a pediatric neurologist with experience in neonatology, the possibility of performing electroencephalography (EEG) with an integrated amplitude or an early conventional EEG, a very early magnetic resonance imaging (MRI) examination and laboratory tests (umbilical cord blood gases, creatine phosphokinase – CPK, lactic dehydrogenase – LDH, and others) are not available in the first hours of life or even afterwards, especially in poor or developing countries.

For the implementation of this clinical score it is important to train the medical and paramedical teams, as well as to print and distribute a copy of the score to all team members, and posting it in all sectors of the neonatology unit.

Validation of the score

No one argues that clinical assessment is essential in neonatology, and our score adds the possibility of a better clinical assessment of newborns from the delivery room onwards. To date, we have used this score in three neonatal units to assess 26 newborns with a gestational age of over 35 weeks and suspected diagnosis of HIE while in the delivery room. All of these newborns had an Apgar score 1' ≤ 3 and presented a Siben score of 3 or more points in the delivery room after completion of the Apgar score, or were sent to the neonatal intensive care unit (NICU) with strong suspicion of HIE. The diagnosis was confirmed in all newborns based on the development of the clinical

TABLE 1 Siben Neurological Score.

HIE	Level of consciousness	Spontaneous activity	Posture	Tonus	Suction	Moro reflex	Pupils	Heart rate	Breathing	Convulsion	Total points
Mild	Hyperalert	Normal	Mild distal flexion	Normal	Weak	Strong	Mydriasis	Tachycardia	Spontaneous	Absent	
Moderate	Lethargy	Decreased	Marked distal flexion	Hypotonia	Weak or absent	Weak	Miosis	Bradycardia	Periodic	Frequent	
Severe	Stupor/Coma	Not present	Decerebration	Flaccidity	Not present	Not present	Diverted/ Non-reactive	Variable	Apnea	Infrequent	

HIE: hypoxic ischemic encephalopathy.

condition (emergence of seizures, apnea, bradycardia or tachycardia, pupil changes, change in tonus, and eventual death) and all showed high levels of CPK and LDH. All of the newborns underwent therapeutic hypothermia. Seven newborn babies progressed to death (27%) and the remaining 19 underwent MRI after 7 days of life. Six infants did not show any alterations in the MRI examination, ten infants had some degree of damage to the basal ganglia and three newborns had damage to the gray matter. The alterations presented by these 13 newborns were compatible with the diagnosis of hypoxic ischemic insult with subsequent progression to HIE (Table 2).

All of the assessors were unanimous in reporting ease of use from the delivery room onwards and in the sequential neurological assessment of the newborn, greatly facilitating the diagnosis of HIE and progressive HIE, in which the patient shows progressive clinical neurological deterioration that can progress from mild to serious symptoms in a few hours.[12] It is very likely that in every center in which this clinical score is implemented there will be no more undiagnosed cases, with at least a suspected diagnosis of HIE, and we can use therapeutic measures sooner, including therapeutic hypothermia, preventing delayed diagnosis and administration of the appropriate therapy. The next step in the evaluation and validation will be to compare the statistics and the monitoring of patients managed using this score.

With the advances that have occurred in recent years to improve neonatal outcomes in newborns with HIE, the establishment of an algorithm for the management of HIE is fundamental, in addition to prevention and early diagnosis, as described below.

HIE management algorithm

Seeking to facilitate the management of newborns with suspected HIE and/or diagnosed with HIE, we have developed an algorithm to guide the management of newborns from the delivery room onwards in different clinical situations.

The importance of the Siben score for HIE lies in the fact that it is a clinical score that is easy to conduct. Below, we will describe an algorithm with measures to evaluate and manage newborns based on their resulting score. Obviously, the need for resuscitation with or without cardiac massage, the need for access to central vessels, mechanical ventilation, and drugs will be undertaken according to the general conditions of the newborn and according to the protocols used, as well as the provision of the general basic required by the newborn. The pH and/or excess base obtained in the umbilical cord blood or soon after birth may be useful, but not always available. A pH < 7.1 and/or BE > -14 has an acceptable positive predictive value; however, they are not pathognomonic of HIE. A pH and/or BE close to normal does not have negative predictive value. Therefore, if the clinical score demonstrates moderate or severe degree,

TABLE 2 Clinical alterations compatible with HIE.

	n (%)	Magnetic resonance imaging with more than 7 days of life (n=19)	n (%)
Lethargy	18 (69.2%)		
Stupor/Coma	5 (19.2%)		
Mild distal flexion	9 (34.6%)	Normal	6 (31.5%)
Marked distal flexion	12 (46.15%)		
Decerebration	5 (19.2%)		
Hypotonia	16 (61.5%)	Changes in the basal ganglia	10 (52.63%)
Flaccidity	10 (38.4%)		
Weak suction	18 (69.2%)	Alterations to the gray matter	3 (15.78%)
No suction	8 (30.7%)		
Weak Moro reflex	19 (73.07%)		
No Moro reflex	7 (26.9%)		
Pupils mydriasis/miosis	20 (76.9%)		
Non-reactive pupils	6 (23.07%)		
Bradycardia	14 (53.8%)		
Bradycardia/Tachycardia	12 (46.15%)		
Periodic respiration	20 (76.9%)		
Apnea	6 (23.07%)		
Convulsions	14 (53.8%)		

HIE: hypoxic ischemic encephalopathy.

this should not be ignored even with a pH > 7.1. Similarly the CPK and LDH dosages have no prognostic value and are not useful, in isolation, for a therapeutic decision. Furthermore, the laboratory results of enzymes may not be available or it may take several hours to obtain these results.

The flowchart can guide the management of newborns with clinical symptoms compatible with that described, facilitating treatment (Figure 1).

Treatment

To prevent hypocapnia (cerebral hypoflow) or hypercapnia (cerebral hyperflow) during mechanical ventilation. To prevent hyperthermia (accelerates the phenomenon of neuronal apoptosis) and hyperoxemia (causes oxidative stress), increasing the risk of neuronal damage. To correct any occasional metabolic disturbances (hypocalcemia, hypoglycemia, and hypomagnesemia) that can worsen the clinical condition.

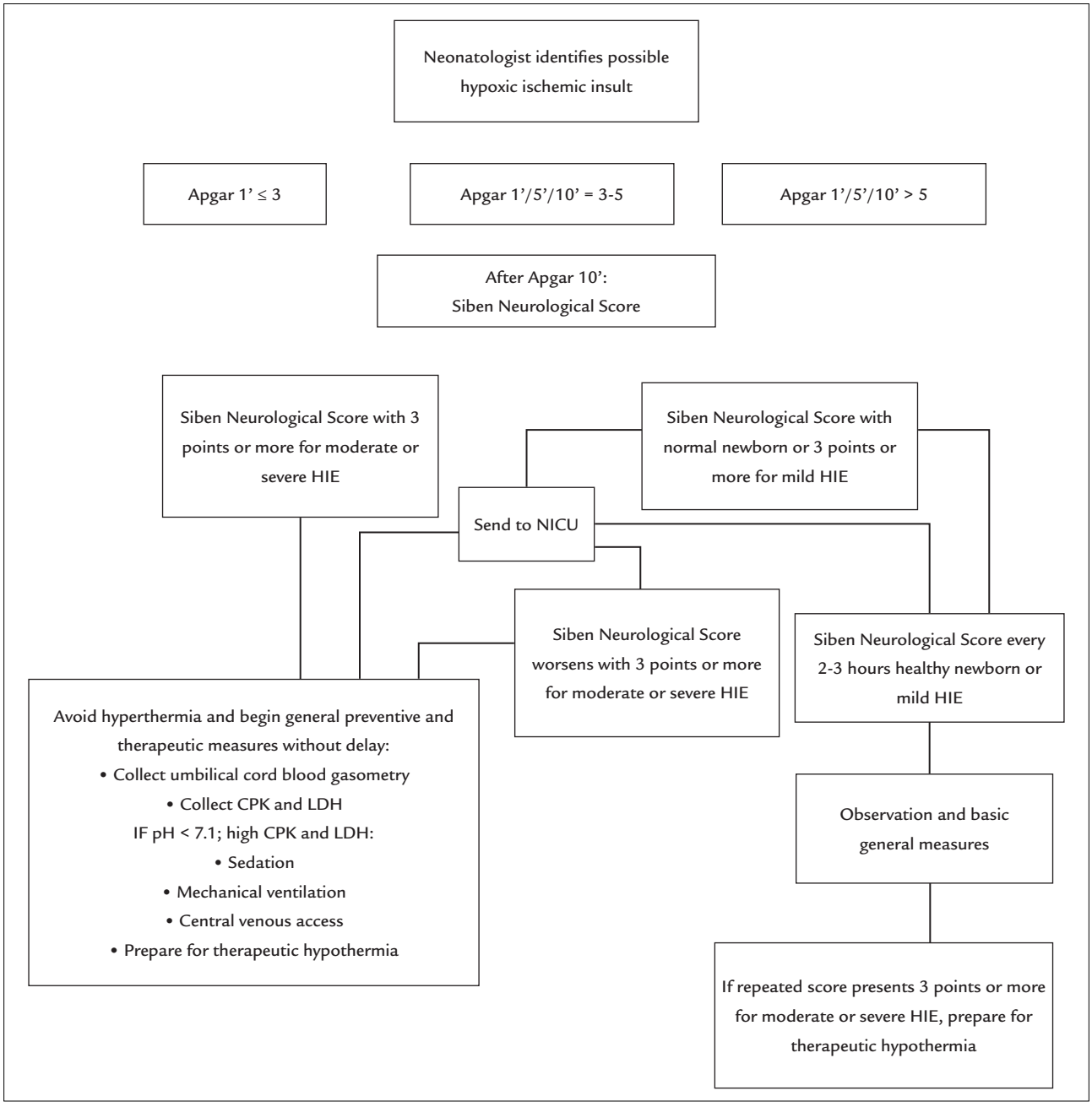

FIGURE 1 The flowchart summarizes the basic preventive and therapeutic measures of the Siben Neurological Score.
HIE: hypoxic ischemic encephalopathy; CPK: creatine phosphokinase; LDH: lactic dehydrogenase; NICU: neonatal intensive care unit.

Therapeutic hypothermia is a therapeutic strategy with scientifically proven benefits. However, the disparity of the effectiveness of this therapy in developed and poor and/or developing countries is clear,[13] demonstrating advantages in the use of equipment with Servo control and the supply of heat by convection.[14]

In three studies already published,[15,16] we have presented an alternative to the use of a device developed in São Paulo (Brazil), the Neonatflow, with Servo control and supply of heat by convection, as well as humidity and isolation through the laminar flow system. We believe that this device facilitates the treatment of these patients, especially in poor and/or developing countries, where the incidence of HIE is highest.

The basic measures to be adopted in a patient with a diagnosis of moderate or severe HIE based on the Siben score will initially be to avoid hyperthermia, by turning off the radiant heat cradle or the incubator or adjusting the desired central temperature by servo control if using the Neonatflow in order to maintain the central temperature between 33-34°C for 72 hours. With regard to the ventilatory part, prevent hypoxia and hyperoxia, hypocapnia and hypercapnia, promote continuous sedation through central venous access, bladder catheterization and installation of the EEG, if available.

Conclusion

The use of this clinical score can improve the objectivity of the assessment and monitoring of newborns and the early start of treatment. The use of the Siben Neurological Score proved to be easy to implement and provided a more objective and early diagnosis of HIE. It may be of greater value in poor and/or developing countries, or in neonatal units without access to high-cost diagnostic examinations (imaging, laboratory, and others).

Conflict of interest

José Maria Rodriguez Perez is one of the patent holders of the Neonatflow device.

Resumo

Escore clínico de encefalopatia hipóxico-isquêmica da Sociedad Iberoamericana de Neonatología (Siben): uma nova proposta para seu diagnóstico e manejo perinatal

A encefalopatia hipóxico-isquêmica é a principal complicação da asfixia perinatal, com alta morbidade, mortalidade e incidência de sequelas neurológicas, como a paralisia cerebral, principalmente em países pobres e/ou em desenvolvimento. Nessas regiões, as dificuldades no diagnóstico e no manejo desses recém-nascidos é surpreendente, o que resulta em dados pouco confiáveis e em péssimos desfechos tanto no que se refere à mortalidade como à incidência de sequelas neurológicas. O objetivo deste artigo é apresentar um novo escore para o diagnóstico clínico ser iniciado na sala de parto e uma abordagem terapêutica com o intuito de melhorar esses resultados.

Palavras-chave: asfixia, encefalopatia hipóxico-isquêmica, Escore Neurológico Siben.

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A "miracle" cancer drug in the era of social media: A survey of Brazilian oncologists' opinions and experience with phosphoethanolamine

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Summary

Introduction: Patients who are treating cancer have often used alternative therapies. In the internet era, information can be broadcasted widely, and this happened with phosphoethanolamine in Brazil, where this substance was claimed by the population to be the "cure for cancer."
Method: This is a cross-sectional study developed by the Brazilian Society of Clinical Oncology (SBOC). An objectively structured questionnaire was sent by e-mail and SMS to active MDs members of the SBOC. Descriptive statistics was used to evaluate the data. Statistical significance between the variables was tested by Pearson's Chi-squared test ($p<0.05$ was considered significance).
Results: The survey was sent to 1,072 oncologists, and 398 (37.1%) answered at least part of it. One hundred and fifteen (28.9%) had followed patients who had used phosphoethanolamine. Among these, 14 (12.2%) observed adverse events and four (3.5%) attributed clinical benefit to the substance. Most of the oncologists (n=331; 83.2%) believe that it should only be used as part of a clinical trial protocol. Most physicians did not recommend this drug to their patients (n=311; 78.1%). Oncologists in Southeast, South and Midwest Brazil were more likely to have patients taking the drug compared to the Northern and Northeastern regions.
Conclusion: This is the first survey to assess the opinion and experience of oncologists about this alternative therapy. Most oncologists in Brazil do not believe that synthetic phosphoethanolamine is active in cancer treatment, do not recommend its use without proper evaluation, and state that it should only be available to patients in the context of clinical trials.

Keywords: phosphoethanolamine, alternative therapies, cancer, Brazil.

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Introduction

Roughly 14.1 million new cases of cancer and 8.2 million deaths occurred worldwide in 2012.[1] New drugs and treatments have started to improve outcomes in high-income nations as well as in low- and middle-income countries. Many alternative therapies, some even called "miracle therapies," have risen in every part of the world and a few cancer hoaxes exist that are older than the discipline of medical oncology itself. However, rarely we have had the opportunity to observe and document the appearance and development of an alternative treatment in real time as we now do for phosphoethanolamine in Brazil.

As social media has become more widely available, unreliable and untruthful information can be broadcasted to an even wider audience. In Brazil, it was social media that made a case for phosphoethanolamine and took the country by storm. Phosphoethanolamine is a precursor of the biosynthesis of phospholipids in the

cellular membrane and part of the cell signaling system either directly or via second messengers. A chemistry professor and his team in one of the campuses of the University of São Paulo have studied the drug in its synthetic version. Potential antineoplastic effects have been demonstrated in a few preclinical studies using cell lines[2-5] and mice models;[6,7] nonetheless, no appropriate safety studies have been carried out in humans. That has not kept the research team from manufacturing the substance in one of the chemistry laboratories at the university and distributing it to an increasing number of patients over the years. In 2015, after the university administration stopped this unsupported practice, patients who had been receiving the substance filed for court order to guarantee continued access to it.

In October 2015, one of the cases reached a Brazilian Federal Court judge, who ruled that patients could continue using phosphoethanolamine and that the university laboratory should not stop making it. Following this decision, additional lawsuits were brought forward. In the same month, a hearing in the National Congress demonstrated the commotion of patients who placed all their hope in the supposed drug and clamored for action. After this meeting, the Ministry of Health created and funded (to the tune of US$2.5 million) a task force to conduct preclinical studies and clinical trials to evaluate phosphoethanolamine as a cancer treatment.

The upheaval and pressure from groups of patients and family members have been so forceful and misguided that legislators have approved a path for the distribution of phosphoethanolamine even before adequate studies were done and evaluated by the Brazilian drug authority, Anvisa (Agência Nacional de Vigilância Sanitária, in portuguese), which in conjunction with the Ministry of Health and medical groups are now asking the president to veto the bill.

As there are no clinical data available on the use of phosphoethanolamine, we designed this survey of medical oncologists in Brazil not only to evaluate their experience with the substance, but also to assess their knowledge, opinion and attitudes on this unfolding story.

Method

Study design

This was an official study designed and carried out by the Brazilian Society of Clinical Oncology (SBOC). We designed a cross-sectional study and developed a survey, which was sent three times by e-mail and three times by text message in 3 to 5-day intervals to active MDs member of the SBOC. A questionnaire with closed-ended questions was applied. The main issues were: how many patients used phosphoethanolamine, how many wished to use it, if any side effects or benefits were seen in those who used it, what was the oncologist's opinion and what recommendation was given to the patient regarding phosphoethanolamine, how many studies the doctor had access to, what would be their position in relation to the distribution of phosphoethanolamine, what was their opinion on the conduct of clinical studies, and the degree of agreement with the opinion of the representatives of SBOC. Data collected between December 11th and 18th, 2015, included demographic information and the physicians' knowledge and opinions regarding the use of phosphoethanolamine in cancer treatment.

Statistical analysis

We used descriptive statistics to evaluate and present the data gathered.

Contingency tables were used to associate the sample characteristics (region where respondent practices, gender and field of practice) to the provided answers regarding phosphoethanolamine. Statistical significance between the variables was tested by Pearson's Chi-squared test.

Data was processed using statistical software PASW, version 18. In all statistical tests, a two-tailed level of 5% of significance ($p<0.05$) was considered.

A formal sample size computation was not performed because this was an exploratory cross-sectional study where the more responses we had, the more information we would gather about the physicians' experience with phosphoethanolamine. Therefore, we aimed to gather the maximum respondents among all current 1,072 active members of the SBOC.

Results

Characteristics of the respondents

The survey was sent to all oncologists who were active members of the SBOC (1,072 doctors), of which 398 (37.1%) answered at least part of it. Among responders, 209 (52.5%) practice medicine in the Southeastern region, 67 (16.8%) in the Northeastern region, 71 (17.8%) in the Southern region, 34 (8.5%) in the Midwestern region and eight (2%) in the Northern region (Figure 1). Nine doctors (2.3%) did not answer which region they were from. 104 (26.1%) of the individuals were men and 49 (12.3%) were women (61.6% of respondents did not answer this question).

As for the setting of practice, 170 (42.7%) oncologists perform their activities in both the public and private sectors, 163 (41%) work predominantly in the private sector and 64 (16.1%) predominantly in the public sector.

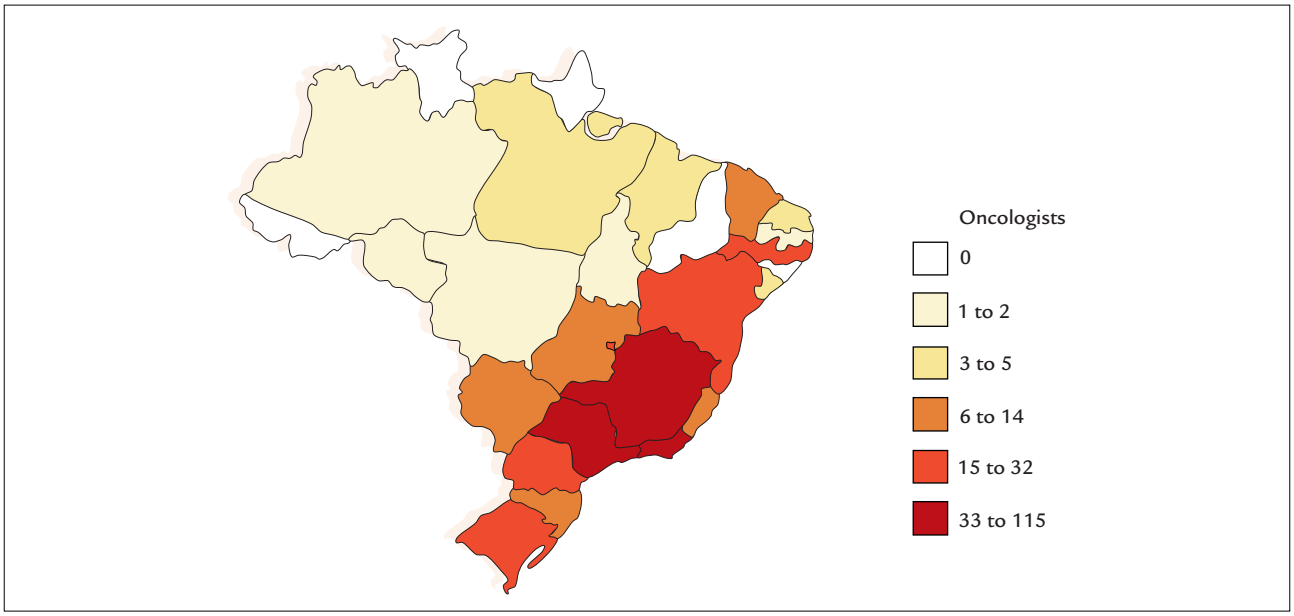

FIGURE 1 Distribution of oncologists by state who answered the survey.

One hundred and fifteen (28.9%) oncologists had followed patients who had used or were using phosphoethanolamine, 93 of whom (80.9%) had up to five patients in this scenario. Among them, only 14 (12.2%) physicians observed any adverse events and four (3.5%) attributed some clinical benefit to the treatment. A total of 378 (95%) respondents stated that they had had patients who wished to use this substance.

As for the recommendation given to patients about the use of phosphoethanolamine, 198 (49.7%) oncologists did not recommend its use, 113 (28.4%) contraindicated it, 42 (10.6%) accepted its use as a complementary treatment as long as another prescribed treatment with an approved drug was maintained, and 15 (3.8%) accepted it depending on the circumstances.

Overall, 299 (75.1%) respondents had not read any scientific studies on phosphoethanolamine, 94 (23.6%) had read between one and five preclinical studies, four (1%) had read five to ten, and one (0.3%) individual answered that he or she had read more than ten studies.

One hundred and fifty four oncologists (38.7%) did not have any informed opinion regarding the drug, and of these, 112 (72.7%) thought it was likely to be well-tolerated. About a quarter of respondents (96, 24.1%) were not convinced of its efficacy despite considering that phosphoethanolamine is likely to be well-tolerated, 31 (7.8%) believed it to be not active and likely to have relevant adverse effects, and two (0.5%) considered it as likely to be an active drug.

As for patient access to phosphoethanolamine, 331 oncologists (83.2%) defended the position that it should only be provided to patients in the context of clinical trials, 26 (6.5%) that it could be considered off protocol, since there were no potentially approved treatments, and two (0.5%) said that it should be administered to any patient who asks for it. The others had either no informed opinion or did not answer.

When questioned if clinical trials probing phosphoethanolamine were a priority, 113 (28.4%) of the respondents agreed, whereas 197 (49.5%) shared the opinion that testing is necessary but not a priority. Approximately two thirds of oncologists (n=267; 67.1%) completely agreed with the position of the SBOC representatives in their public opinions, whereas 36 (6%) partly agreed. However, 86 oncologists (21.6%) had not seen the interviews nor read the articles in which the society stated its views that adequate clinical trials and evaluation by Anvisa should be undertaken before the substance becomes available to the public.

These results are detailed in Tables 1, 2 and 3.

Chi-square tests

No significant associations were observed between site of practice (private and/or public sector) and any of the answers to the survey.

There was a significant association between region and access to phosphoethanolamine. Oncologists in the Southeast, South, and Midwest were more likely to have patients

TABLE 1 Medical oncologists' experience with phosphoethanolamine.

Questions about phosphoethanolamine		Region where the respondents reside									p-value
		Brazil	North-Northeast		Midwest		Southeast		South		
		N*	n	%	n	%	n	%	n	%	
How many patients under your care used or are using phosphoethanolamine?	None	276	61	81.3	20	58.8	146	69.9	49	69.0	**.018***
	1 to 5 patients	92	14	18.7	12	35.3	52	24.9	14	19.7	
	5 to 10 patients	13	0	0	2	5.9	8	3.8	3	4.2	
	More than 10 patients	8	0	0	0	0	3	1.4	5	7.0	
How many patients under your care expressed the desire of using phosphoethanolamine?	None	19	1	1.3	0	0	12	5.7	6	8.5	.195
	1 to 5 patients	116	26	34.7	9	26.5	63	30.1	18	25.4	
	5 to 10 patients	73	20	26.7	7	20.6	35	16.7	11	15.5	
	More than 10 patients	181	28	37.3	18	52.9	99	47.4	36	50.7	
In the follow-ups, did you observe any adverse effect that you attributed to phosphoethanolamine?	No	95	10	13.3	10	29.4	50	23.9	15	21.7	.344
	I did not follow patients with phosphoethanolamine	288	63	84.0	24	70.6	150	71.8	51	73.9	
	Yes	14	2	2.7	0	0	9	4.3	3	4.3	
Did you consider any benefit that you attributed to the treatment?	No	98	12	16.0	9	26.5	56	26.8	21	30.4	.421
	I did not follow patients with phosphoethanolamine	285	62	82.7	25	73.5	150	71.8	48	69.6	
	Yes	4	1	1.3	0	0	3	1.4	0	0	
How do you advise your patients who are using or wish to use phosphoethanolamine?	I accept the use as long as they do not abandon the prescribed treatment	40	3	4.0	7	20.6	22	10.5	8	11.3	.138
	I contraindicate it emphatically	111	23	30.7	9	26.5	59	28.2	20	28.2	
	Depending on the situation, I support its use	15	2	2.7	1	2.9	10	4.8	2	2.8	
	I do not discuss the matter	7	1	1.3	0	0	6	2.9	0	0	
	None of the above	22	0	0	2	5.9	17	8.1	3	4.2	
	I recommend that they do not use it	194	46	61.3	15	44.1	95	45.5	38	53.5	
How many studies about phosphoethanolamine have you read?	None	290	63	84.0	26	76.5	151	72.2	50	70.4	.696
	1 to 5 studies	94	12	16.0	8	23.5	54	25.8	20	28.2	
	5 to 10 studies	4	0	0	0	0	3	1.4	1	1.4	
	More than 10 studies	1	0	0	0	0	1	.5	0	.0	
Total		**389**	**75**	**100.0**	**34**	**100.0**	**209**	**100.0**	**71**	**100.0**	

Note: The statistical significance (p-value) refers to Chi-Square test.
*Nine doctors (2.3%) did not answer these questions.

TABLE 2 Medical oncologists' opinions on phosphoethanolamine.

Questions about phosphoethanolamine		Region where the respondents reside									p-value
		Brazil	North-Northeast		Midwest		Southeast		South		
		N*	n	%	n	%	n	%	n	%	
What is your opinion about phosphoethanolamine?	I believe that it is not active and that it causes significant adverse events	30	6	8.0	1	2.9	16	7.8	7	9.9	.835
	I believe that it is not active, but believe it is likely to be well-tolerated	109	18	24.0	15	44.1	55	26.7	21	29.6	
	I am convinced of its efficacy and that it is well-tolerated	1	0	0	0	0	1	.5	0	0	
	I am convinced of its efficacy but I have doubts about its tolerability	1	0	0	0	0	1	.5	0	0	
	I am not convinced of its efficacy but I believe it to be well-tolerated	95	20	26.7	5	14.7	50	24.3	20	28.2	
	I have no opinion about phosphoethanolamine	150	31	41.3	13	38.2	83	40.3	23	32.4	
What is your position regarding the distribution of phosphoethanolamine to patients with cancer?	I do not have enough information to have an opinion	35	6	8.1	2	5.9	22	10.5	5	7.1	.065
	It can be considered for patients with no alternative approved treatment, even outside clinical trials	26	3	4.1	5	14.7	17	8.1	1	1.4	
	It can be considered for any patient	2	0	0	1	2.9	0	0	1	1.4	
	It should only be provided to patients in clinical trials	324	65	87.8	26	76.5	170	81.3	63	90.0	
Total		**387**	**75**	**100.0**	**34**	**100.0**	**209**	**100.0**	**71**	**100.0**	

Note: The statistical significance (p-value) refers to Chi-Square test.
*Eleven doctors (2.3%) did not answer these questions.

TABLE 3 Medical oncologists' attitudes towards clinical trials of phosphoethanolamine and the Brazilian Society of Clinical Oncology statements and position.

Questions about phosphoethanolamine		Region where the respondents reside									p-value
		Brazil	North-Northeast		Midwest		Southeast		South		
		N*	n	%	n	%	n	%	n	%	
What is your opinion about carrying out clinical trials on phosphoethanolamine?	I think they are unnecessary as I am convinced that the substance has no activity	4	3	4.0	0	0	1	.5	0	49.3	**0.093**
	I think they are necessary but should not be a priority	195	38	50.7	20	58.8	102	49.0	35	5.6	
	I do not have enough information to have an opinion	34	11	14.7	3	8.8	16	7.7	4	12.7	
	I am against it, as I believe that preclinical trial data are insufficient to bring it for clinical studies	46	4	5.3	2	5.9	31	14.9	9	32.4	
	Clinical trials are necessary and should be considered a priority	109	19	25.3	9	26.5	58	27.9	23	0	
How strongly do you agree with the position of SBOC representatives in regards to their public display of opinions?	I partly agree	35	4	5.3	6	17.6	17	8.2	8	11.3	.204
	I completely agree	265	55	73.3	22	64.7	139	66.8	49	69.0	
	I partly disagree	6	0	0	0	0	3	1.4	3	4.2	
	I completely disagree	2	1	1.3	0	0	0	0	1	1.4	
	I did not follow the interviews and articles	80	15	20.0	6	17.6	49	23.6	10	14.1	
Would you like to see another position defended by SBOC?	No	226	45	60.8	23	67.6	116	56.0	42	60.0	.570
	I did not follow the articles. I would rather not comment	88	19	25.7	6	17.6	51	24.6	12	17.1	
	Yes	71	10	13.5	5	14.7	40	19.3	16	22.9	
Total		**385**	**75**	**100.0**	**34**	**100.0**	**209**	**100.0**	**71**	**100.0**	

Note: The statistical significance (p-value) refers to Chi-Square test.
*Thirteen doctors (2.3%) did not answer these questions.

taking the substance compared with those in the North and Northeast.

Discussion

There is clearly a need for more active therapeutic alternatives in cancer treatment and patients who have no further specific anticancer options may easily fall prey to the use of unproven therapies, especially when unconfirmed and poorly documented cases of response or symptomatic improvement appear in social media. In Brazil, phosphoethanolamine has quickly become the most commented--about alternative treatment.

In this study, we show that almost all oncologists in Brazil have had patients who voiced an interest in phosphoethanolamine and that a little less than one third of physicians in the country have treated individuals who used the substance. Potential clinical benefit, defined as clinical response or symptomatic improvement, was reported by a very small number of respondents (3.5%), as was the incidence of adverse events (reported by only 12.2% of oncologists).

The vast majority of oncologists do not think there is enough evidence for efficacy and do not recommend the drug, stating that it should be only used in the context of a clinical trial. Information about phosphoethanolamine is still very limited: 75% of the oncologists surveyed stated that they had never read any articles evaluating it and around one in five did not follow the interviews and mainstream media articles on the subject. This reflects the complete lack of scientific evidence about the clinical outcomes offered by phosphoethanolamine. The only available literature comprises preclinical studies with cell cultures and animal models where this substance has shown some antitumor activities in solid tumors, with the most recent one involving renal cell carcinoma.[5]

A significant difference in the number of oncologists who followed patients using phosphoethanolamine in different regions of the country was observed. This is likely to be due to access to the molecule, which is produced in São Paulo, in the Southeastern region of Brazil, therefore making it more accessible in this area and in the adjacent South and Midwest of Brazil.

It is clear from the short time of it took us to enroll the planned sample in this study (only one week) that there is strong interest within the medical community; congressional hearings and the approved bill creating a path for the distribution of phosphoethanolamine also attest to the public's interest in this matter. SBOC and its members strongly believe, however, that adequate clinical trials are of paramount importance and absolutely needed before the substance can be considered in the treatment of patients with cancer. Furthermore, the uncontrolled distribution and use of phosphoethanolamine without proper evidence of its benefits and safety constitute a hazard to public health in Brazil.

Cancer patients often take alternative compounds. Systematic reviews have reported that 40 to 90% of patients with breast or gynecology cancers have taken alternative medicines while on oncology treatments or follow-up.[8] In this context, several supposedly "miracle drugs" such as herbs, vitamins, and fruit extracts, among others, have appeared in different countries. Commotion is expected within the society because cancer patients and their relatives are desperate to try anything that could cure or prolong their lives. Financial interests have led to many unproven compounds to be sold to cancer patients worldwide. One example is that of Sun Mushroom (*Agaricus*), which is native to Brazil and was used in the past for virtually all medical conditions, including cancer, due to its pre-clinical immunomodulatory effects.[9] Despite not being formally tested in clinical trials, it was used off-label in Brazil by many cancer patients. While the clinical effects of this mushroom have never been published, adverse events were observed in some patients, such as severe hepatic dysfunction. This example stresses how crucial it is to formally test new compounds in well-designed clinical trials.

This study has some limitations, the main one being a possible selection bias. Only active members of the SBOC were invited to answer the survey and 37.1% of them answered the questionnaire. However, given that this is the first survey about the experience with phosphoethanolamine and there are no clinical trials available studying the use of this substance, it represents the best available evidence for its use. Detailed information about tumor types and setting were not collected, which prevented us from evaluating the cases of responsive patients.

We expect that society, lawmakers, and the judicial system will reach the understanding that drug development is a scientific matter and redirect their efforts into improving and expediting our regulatory system for clinical trials and drug approval, speeding up the availability of truly promising drug candidates and proven medications to our patients.

Conclusion

To our knowledge, this is the first survey to assess the opinion and experience of oncologists during the early stages of use of a supposed "miracle" alternative therapy. The results show that most oncologists in Brazil do not believe that synthetic phosphoethanolamine is active in the treatment of cancer, do not recommend its use without proper evaluation, and state that it should only be available to patients in the context of clinical trials.

Resumo

Uma "milagrosa" droga contra o câncer na era da mídia social: uma pesquisa sobre a opinião e a experiência dos oncologistas brasileiros com a fosfoetanolamina

Introdução: Alguns pacientes com diagnóstico de câncer utilizam terapias alternativas. Na era da internet, as informações podem se dissipar de forma rápida e abrangente, como foi o caso da fosfoetanolamina no Brasil, onde foi aclamada pela população como sendo a "cura para o câncer". **Método:** Trata-se de um estudo transversal desenvolvido pela Sociedade Brasileira de Oncologia Clínica (SBOC). Através de *e-mail* e SMS, enviou-se um questionário com perguntas objetivas para oncologistas membros ativos da SBOC. Os dados foram avaliados por meio de estatística descritiva. A significância estatística entre as variáveis foi

testada pelo teste Qui-quadrado de Pearson (p<0,05 foi considerado significativo).
Resultados: O questionário foi enviado para 1.072 oncologistas, tendo 398 (37,1%) respondido pelo menos parte dele. Cento e quinze (28,9%) tinham pacientes que fizeram uso da fosfoetanolamina. Desses, 14 (12,2%) observaram eventos adversos e quatro (3,5%) atribuíram benefício clínico para a substância. A maioria (n=331; 83,2%) acreditava que ela só deveria ser utilizada dentro de um ensaio clínico. A principal recomendação dada aos pacientes foi contra o seu uso (n=311; 78,1%). Oncologistas das regiões Sudeste, Sul e Centro-Oeste tiveram mais pacientes que tomaram a substância quando comparados com as regiões Norte e Nordeste.
Conclusão: Este é o primeiro estudo que avalia a opinião dos oncologistas sobre essa terapia alternativa e sua experiência. A maioria dos oncologistas brasileiros não acredita que a fosfoetanolamina sintética seja ativa no tratamento do câncer, não recomendando seu uso sem avaliação adequada, e afirmam que a substância só deve estar disponível no contexto de ensaios clínicos.

Palavras-chave: fosfoetanolamina, terapias alternativas, câncer, Brasil.

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Zinc and metalloproteinases 2 and 9: What is their relation with breast cancer?

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Summary

Zinc is the catalytic component of proteins that regulate responses to DNA damage, intracellular signaling enzymes, and matrix metalloproteinases, which are important proteins in carcinogenesis. The objective of this review is to bring current information on the participation of zinc and matrix metalloproteinases types 2 and 9 in mechanisms involved in the pathogenesis of breast cancer. We conducted a literature review, in consultation with the PubMed, Lilacs, and Scielo databases. The zinc and cysteine residues are structural elements shared by all members of the family of matrix metalloproteinases, and these proteins appear to be involved in the propagation of various types of neoplasms, including breast cancer. Moreover, transported zinc is likely to be used for the metalation of the catalytic domain of the newly synthesized metalloproteinases before the latter are secreted. Accordingly, increase in zinc concentrations in cellular compartments and the reduction of this trace element in the blood of patients with breast cancer appear to alter the activity of metalloproteinases 2 and 9, contributing to the occurrence of malignancy. Thus, it is necessary to carry out further studies with a view to clarify the role of zinc and metalloproteinases 2 and 9 in the pathogenesis of breast cancer.

Keywords: zinc, matrix metalloproteinases, breast neoplasms.

Introduction

Breast cancer is a multifactorial disease, mainly determined by the occurrence of mutations or abnormal activation of genes that control cell growth and proliferation.[1] The mechanisms involved in the genesis of the disease are not yet fully understood. However, it is known that there is an interaction between genetic and environmental factors, and certain nutrients have important roles in the inhibition of cancer or in its development.[2]

Studies have demonstrated the role of micronutrients in anticarcinogenic mechanisms.[3] Zinc, in particular, has been a nutrient of great interest, given that it is a catalytic component in more than 300 enzymes, including those involved in antioxidant defense, for example, metallothionein and Cu/Zn superoxide dismutase.[4]

Zinc also acts as a transcription factor of enzymes involved in the synthesis of DNA and RNA and as a cofactor of proteins that control responses to DNA damage, intracellular signaling enzymes, and matrix metalloproteinases (MMPs), which are proteins involved in the pathogenesis of breast cancer.[5] As such, changes in zinc concentrations may play a significant role in cell dysfunction and proliferation, including the development and progression of this disease.[2]

Gelatinase class MMPs, such as type 2 (MMP-2) and 9 (MMP-9) metalloproteinases, have the ability to degrade collagen IV that makes up the basal lamina, and are probably relevant in the acquisition of the invasive phenotype of malignant neoplasms.[6]

Considering the changes in zinc metabolism in patients with breast cancer and the important relation be-

tween this mineral and MMPs in the progression of this disease, this review intends to provide up-to-date information about the role of zinc and matrix metalloproteinases 2 and 9 in mechanisms involved in the pathogenesis of breast cancer.

Method

A bibliographic search was conducted on the PubMed, Scielo, and Lilacs databases, without any limit on the year of publication, considering the following inclusion criteria: studies that examined the relation between zinc concentrations and the expression of MMPs in patients with breast cancer. The articles were selected in relation to their originality and relevance, considering the rigor and adequacy of the experimental design and the sample number.

The search for bibliographic references was carried out using the following keywords: "zinc," "matrix metalloproteinases," and "breast neoplasms." The bibliographic search covered the following types of studies: randomized or quasi-randomized controlled clinical trials, in vitro studies, case-control studies and review articles.

Zinc and metalloproteinases of the extracellular matrix

MMPs are a family of more than 25 species of proteases that rely on zinc for their catalytic action and are essential for normal tissue remodeling. Metalloproteinases are able to degrade most components of the extracellular matrix and basal membrane, for example, collagen, elastin, and fibronectin. They can also degrade other proteins that are not characteristic of the extracellular matrix, such as growth factors, cytokines, chemokines, and cell surface receptors.[7]

MMPs are classified into five groups: interstitial collagenases (MMP-1, MMP-8, MMP-13 and MMP-18), which cleave fibrillar collagen types I, II and III; gelatinases (MMP-2 and MMP-9), which degrade amorphous collagen and fibronectin; stromalysins (MMP-3, MMP-10 and MMP-11), which act on a variety of extracellular matrix components, including proteoglycans, laminin, fibronectin, and amorphous collagen; membrane type MMPs (MMP-14, MMP-15, MMP-16, MMP-17, MMP-24 and MMP-25), which are proteases associated with the cell surface; and the matrilysins (MMP-7, MMP-12, MMP-20 and MMP-26), which also degrade laminin, fibronectin, and non-fibrillar collagen.[8]

Two structural elements are shared across all members of the family of MMPs. The first is zinc ion, located at the catalytic site of the protein, and necessary for its action; the second element is propeptide, which contains a cysteine residue. These proteases are secreted in the form of a latent precursor or zymogen, usually called pro-MMP, which is activated in the extracellular space.[9]

The activity of MMPs is regulated in several steps, which include transcription, secretion, and activation through proteolytic cleavage, as well as inhibition via endogenous tissue inhibitors of metalloproteinases (TIMPs). Associated with this, it should be noted that certain proteins can stimulate the expression of MMPs, such as EMMPRIN, a plasma membrane-bound glycoprotein that is involved in inflammation and immune response.[10,11]

Gelatinases, in particular, act on various types of extracellular substrates and are important in many biological processes, with the expression of MMP-2 generally constitutive, while MMP-9 may be induced by tumor necrosis factor α (TNF-α), which includes the activation of nuclear factor kappa β (NF-κβ), mitogen-activated protein kinases (MAPK), phosphatidylinositol-3 kinase, and signaling by protein kinase B.[12,13]

In the remodeling process of the extracellular matrix, macromolecules are secreted and form complex protein networks, some of which are specialized in degrading extracellular proteins, contributing to tissue modifications. The remodeling and tissue regeneration process involves the modulation of enzyme mechanisms, which keep the degradation of extracellular matrix harmonious and balanced in several physiological events. Thus, loss of this modulation is deleterious to the functions and structural stability of the tissues, favoring the emergence of pathological processes.[8]

Extracellular matrix metalloproteinases, zinc, and breast cancer

MMPs seem to be involved in the propagation of various types of neoplasms, including breast cancer. In this regard, studies have verified changes in the expression and increased proteolytic activity of these enzymes in invasive and metastatic tumors.[10] The activity of MMPs in the propagation of the tumor is both direct and indirect. In the first case, it promotes the proliferation of neoplastic cells and metastatic dissemination through the degradation of the extracellular matrix and basal membrane, while indirectly it promotes angiogenesis, providing nutrition and dissemination of the tumor.[14]

The first evidence of the involvement of MMPs in cancer came from studies in animal models. Experiments with tumor cells in mice showed that benign tumor cells acquire malignant properties when there is increased expression of these enzymes. On the other hand, it has been verified that when the expression or activity of MMPs is reduced, the malignant cells become less aggressive.[15]

The first role attributed to MMPs in cancer was the induction of metastasis or, more precisely, the creation of a pathway in the extracellular matrix through which the tumor cells pass to colonize distant tissues.[16] However, it has been verified that these enzymes may also change cell proliferation, adhesion, and migration, not only by degrading the extracellular matrix but also by favoring the release of growth factors and the generation of certain cleavage fragments or functional proteins in this space.[17]

Some MMPs are synthesized by tumor cells, such as MMP-7, while others are produced predominantly by stromal cells, including MMP-2 and 9. Tumor cells can also stimulate the surrounding stromal cells and synthesize these enzymes in a paracrine manner via the secretion of interleukins, interferons, EMMPRIN (extracellular inducer of MMPs), and growth factors.[18] MMP-2 and 9 may also be recruited for the membrane of tumor cells in breast cancer.[19,20]

The literature has shown that there is an association between the expression of MMP-9 and a worse prognosis in breast tumors.[21] The first steps of tumor cell invasion, detachment, and migration are influenced by MMPs. The cleavage of laminin-5 by MMP-2 and MMP-14 reveals its matricryptic site (hidden forms of extracellular matrix molecules that can be exposed by structural modifications), favoring cell motility.[22]

Patel et al.[23] evaluated the expression of MMP-2 and 9 and the plasma concentration of these gelatinases in tissue samples with malignant lesions. The authors suggested that the expression of these MMPs could be a useful diagnostic marker in the detection and monitoring of malignant lesions, given that tissue expression of these enzymes was shown to be high in these lesions and in the plasma. Table 1 shows studies that have assessed metalloproteinases in patients with breast cancer.

Zucker et al.[29] demonstrated that the high expression of MMPs, observed mainly in cancerous processes, is due to changes in gene transcription, with the increase of these enzymes leading to a poor prognosis for various types of cancer.[30,31]

A study conducted by Benson et al.[32] evaluated the expression of MMPs in different types of tissues with breast cancer and normal tissues. The results showed that MMPs are regulated differentially in tissues with breast neoplasm. In addition to the possible use of these enzymes as diagnostic markers, the authors highlight their potential as a pharmacological target because they have different substrate specificities that are regulated during the progression of breast cancer, which are important in tumor invasion, metastasis, and angiogenesis.

MMP-9 plays a vital role in the angiogenesis of tumor as it controls the bioavailability of vascular endothelial growth factor (VEGF), which is a potent inducer of an-

TABLE 1 Studies that have assessed metalloproteinases in patients with breast cancer.

Author(s)	Study design	Results
Jinga et al.[24]	Breast tumor in benign and malignant tumor cells	Higher expression of MMP-9 in malignant tumors Positive relation between MMP-9 and tumor diameter
Daniele et al.[25]	Women with breast cancer, and healthy women Sentinel lymph nodes	High serum concentrations of MMP-2 and MMP-9 in women with breast cancer compared to healthy subjects High concentrations of MMP-2 and MMP-9 in sentinel lymph nodes with macrometastases compared to micrometastases and non-metastatic cases
Somiari et al.[26]	Groups with breast cancer, benign disease and group with high and low risk of developing breast cancer	Concentration and activity of MMP-2 were significantly lower in low-risk patients compared to participants in the other groups. Breast cancer patients had high concentration of total MMP-9 in comparison with those with benign disease
Vasaturo et al.[27]	MMPs-2, 3, and 9 in patients with carcinoma and fibroadenoma	Expression of MMP-2 was significantly higher in patients with carcinoma compared to patients with fibroadenoma Plasma concentrations of MMPs-2 and 9 showed a direct and significant correlation with the histological grade of the tumor
Čupić et al.[28]	Primary and recurrent carcinomas	High expression of MMP-9 in primary carcinomas Increased expression of MMP-9 in recurrent carcinomas after 24 months

MMP: metalloproteinases; MMP-2: metalloproteinase 2; MMP-9: metalloproteinase 9.

giogenesis. MMP-9, together with MMP-2, activates transforming growth factor β (TGF-β) signaling to promote the invasion of the tumor, angiogenesis and metastasis.[32]

The role of MMPs during the neoplastic invasion consists of the rearrangement of the extracellular matrix components in order to better accommodate cell migration. Thus, deregulation of these enzymes plays an important role in several stages of the development of breast cancer and in activities dependent on zinc binding to the catalytic site.[33] It is likely that zinc transported to the cellular compartments is used for metalation of the catalytic domain of the MMPs.[34]

As such, the increased concentrations of zinc in cellular compartments and the reduction of this trace element in the blood of breast cancer patients seems to change the activity of the MMPs, contributing to the occurrence of malignant tumors. Another important function of zinc is related to the angiogenesis process, as this mineral increases the expression of MMPs, especially under conditions of hypoxia.[35]

A study conducted by Holanda[36] verified that low concentrations of zinc in the plasma and erythrocytes are positively related to increased plasma concentrations of metalloproteinase 2 in patients with breast cancer. In addition, a significant difference was observed between plasma concentrations of MMP-2 and MMP-9 in these patients compared to the control group.

Taylor et al.[37] and Kelleher et al.[38] highlight the implications of deregulation of zinc homeostasis in the pathogenesis of breast cancer. According to these authors, an increase in the expression of transporters of this mineral occurs in cells with malignant tumors, including metallothionein, Zip5, Zip6, Zip7, Zip8, and Zip10, producing an influx of zinc into the neoplastic cells, suggesting that its accumulation inside the breast tumor induces processes dependent on this mineral, for example, the activation of MMPs.[39]

Lue et al.[40] demonstrated that zinc transporter protein denominated LIV-1 belonging to the Zip transporter family was associated with increased MMP-2 and MMP-9 activity in prostate tumor cells. This protein may play a role in both cell growth, by acting as a zinc transporter, as well as the induction of metastasis, by association with matrix metalloproteinases.[41] It is worth mentioning that this transporter protein is also detected in neoplastic breast tissue.[42]

Zip4 also appears to regulate the activity of metalloproteinases. Zhang et al.[43] found that the overexpression of this zinc transporter protein favored increased activity of MMP-2 and MMP-9 and inducers of angiogenesis in pancreatic cancer cell lines, suggesting that Zip4 may mediate tumor growth through angiogenesis, invasion, and metastasis pathways in these cells. These results suggest the possible involvement of zinc transporter proteins in the activity of MMPs. However, there are no studies on the action of these proteins in the activation of metalloproteinases in neoplastic mammary cells.

In relation to metallothionein, Kim et al.[44] found that the overexpression of the 2A form of this protein is associated with the aggressiveness of the mammary carcinoma by inducing cellular migration and invasion and regulating the expression of MMP-9, through the activation of transcription factor AP-1 (activator protein 1) and NF-κβ. The authors also noted that the reduction in the expression of metallothionein-2A completely inhibited tumor migration and invasion in the MDA-MB-231 cell line. Zitka et al.[45] also demonstrated that the activity of MMP-9 in collagen degradation increases in the presence of metallothionein in vitro, causing a similar effect to that promoted by temperature in the activity of this protein (Figure 1).

In addition, another zinc-dependent protein, known as zinc finger 24 (ZNF24), seems to exert regulatory activity on MMP-2. Jia et al.[46] verified that the deletion of the gene that encodes this protein in primary microvascular endothelial cells significantly decreases migration and tumor invasion by reducing the levels of MMP-2 and impairing the signaling of VEGF receptor 2. However, the authors did not observe effects of ZNF24 on the regulators of MMP-2 activity or its tissue inhibitors.

A study conducted by Huang et al.[47] aimed to investigate whether the activity of ellagic acid, a natural polyphenol found in fruits and nuts, has antiangiogenic effects through the inhibition of MMP-2, and whether this inhibition could be reversed with the addition of zinc chloride. This research verified that ellagic acid can indeed inhibit the activity and secretion of MMP-2 in human vascular endothelial cells, probably mediated by inducing the expression of RECK (reversion-inducing cysteine-rich protein with Kazal motifs). The authors also found that the antiangiogenic effects caused by ellagic acid can be reversed by the addition of zinc, demonstrating the important role of this trace element in the angiogenic process of tumors.

Research has shown the ability to inhibit MMPs by certain substances that have an affinity to zinc, such as lactoferrin. In this regard, Newsome et al.[48] demonstrated that lactoferrin exerts an effect on the proteolytic activity of MMP-2 and other MMPs by removing zinc from the active site of these metalloproteins, which was reversed upon the addition of zinc chloride.

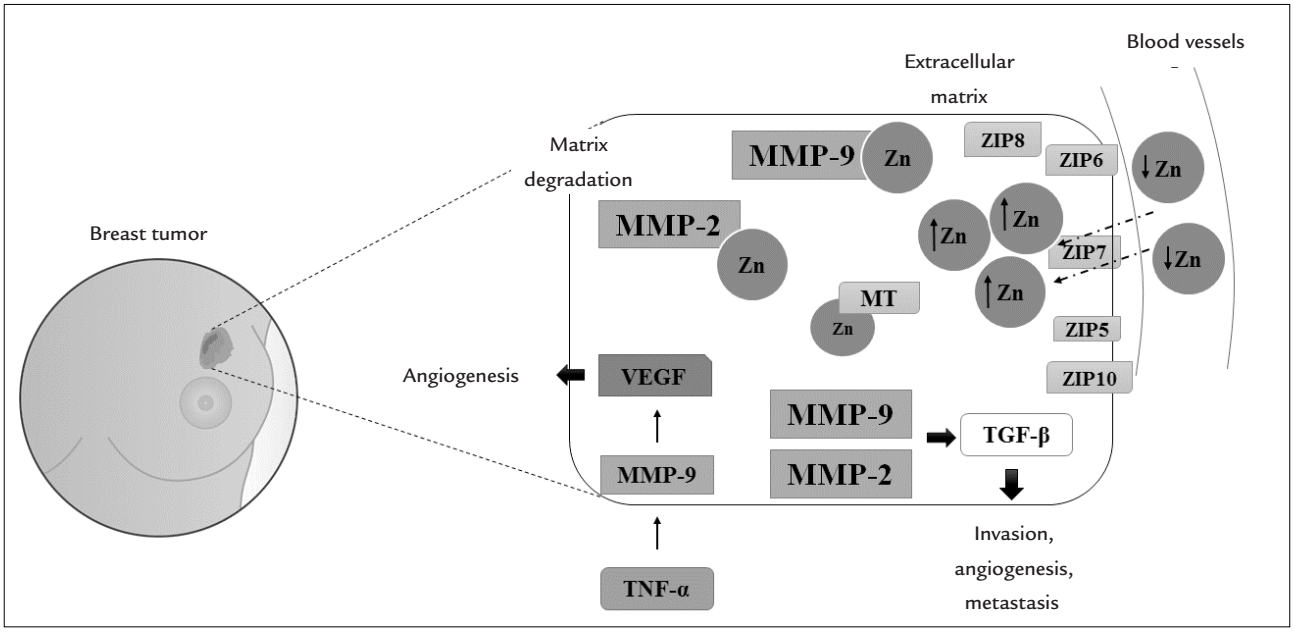

FIGURE 1 Relation between zinc, MMPs, and breast cancer. Zinc compartmentalization within the tumor favors invasion, metastasis, and angiogenesis mediated by matrix metalloproteinases, particularly MMP-2 and MMP-9. The increased zinc in the tumor appears to be due to changes in the expression and activity of its transporter proteins (Zip5, 6, 7, 8, 10, and metallothionein), leading to mineral deficiency in the serum of patients with breast cancer.

MMP-2: matrix metalloproteinase 2; MMP-9: matrix metalloproteinase 9; MT: metallothionein; TGF-β: transforming growth factor β; TNF-α: tumor necrosis factor α; VEGF: vascular endothelial growth factor; Zn: zinc.

Cysteine also appears to inhibit the activity of metalloproteinases due to affinity of zinc for the thiol groups of this amino acid. Therefore, Khrenova et al.[49] observed that the binding of Regasepin 1 to MMP-9 promotes the rearrangement of zinc at the catalytic site of its binding to histidine in order to also bind to two cysteine residues, inhibiting the action of this enzyme. The authors further verified that this drug inhibits MMP-2 because of two replacements in its active site, with cysteine binding.

Thus, considering the complex action of zinc in mechanisms involved in the pathogenesis of breast cancer and its structural role in the activation of MMPs, the inhibition of matrix metalloproteinase activity by removal or chelation of zinc from its active site has been a well-studied therapeutic target in the treatment of cancer. However, new studies that determine the effectiveness of these inhibitors in breast carcinoma are still needed.

Conclusion

There is convincing experimental evidence demonstrating the participation of zinc and matrix MMPs in the pathogenesis of breast cancer. However, although certain mechanisms have been proposed to identify the activity of this mineral and metalloproteinases in the development of tumors, the current information is still quite scarce and inconsistent. It is therefore necessary to carry out further studies on the subject in order to obtain clarification about the influence of the deregulation of zinc homeostasis and the activity of MMPs on the manifestation of breast cancer and its associated disorders.

Conflict of interest

The authors declare no conflict of interest.

Resumo

Zinco e metaloproteinases 2 e 9: qual é a relação com câncer de mama?

O zinco é componente catalítico de proteínas que regulam respostas a danos no DNA, enzimas de sinalização intracelular e metaloproteinases de matriz, proteínas importantes na carcinogênese. O objetivo desta revisão é trazer informações atualizadas sobre a participação do zinco e das metaloproteinases de matriz dos tipos 2 e 9 em mecanismos envolvidos na patogênese do câncer de mama. Realizou-se um levantamento bibliográfico, mediante consulta às bases de dados PubMed, Scielo e Lilacs. O zinco e os resíduos de cisteína são elementos estruturais compartilhados por todos os membros da família das metaloproteinases de

matriz, as quais parecem estar envolvidas na propagação de vários tipos de neoplasias, incluindo o câncer de mama. Além disso, é provável que o zinco transportado seja utilizado para metalação do domínio catalítico das metaloproteinases recentemente sintetizadas antes de serem segregadas. Nesse sentido, o aumento das concentrações de zinco em compartimentos celulares e a redução desse oligoelemento no sangue de pacientes com câncer de mama parecem alterar a atividade das metaloproteinases 2 e 9, contribuindo para a ocorrência de tumor maligno. Assim, faz-se necessária a realização de novos estudos na perspectiva de esclarecer o papel do zinco e das metaloproteinases 2 e 9 na patogênese do câncer de mama.

Palavras-chave: zinco, metaloproteinases da matriz, neoplasias da mama.

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The role of oxidative stress on the pathophysiology of metabolic syndrome

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Summary

Metabolic syndrome (MetS) has a high prevalence around the world. Considering the components used to classify MetS, it is clear that it is closely related to obesity. These two conditions begin with an increase in abdominal adipose tissue, which is metabolically more active, containing a greater amount of resident macrophages compared to other fat deposits. Abdominal adiposity promotes inflammation and oxidative stress, which are precursors of various complications involving MetS components, namely insulin resistance, hypertension and hyperlipidemia. One way to block the effects of oxidative stress would be through the antioxidant defense system, which offsets the excess free radicals. It is known that individuals with metabolic syndrome and obesity have high consumption of fats and sugars originated from processed foods containing high levels of sodium as well as low intake of fruits and vegetables, thus maintaining a state of oxidative stress, that can speed up the onset of MetS. Healthy eating habits could prevent or delay MetS by adding antioxidant-rich foods into the diet.

Keywords: oxidative stress, metabolic syndrome, obesity.

Introduction

Metabolic syndrome (MetS), also known as syndrome X or insulin resistance syndrome, is characterized by the clustering of cardiovascular risk factors such as hypertension, insulin resistance, central obesity, and atherogenic dyslipidemia (high LDL-cholesterol, high triglycerides, and low HDL-cholesterol).[1] MetS is a major health issue of westernized modern societies[1] and it already appears as one of the main challenges of current clinical practice. In general, the International Diabetes Federation (IDF) estimates that one-quarter of the world's adult population has MetS and the observed prevalence of MetS in National Health and Nutrition Examination Survey (NHANES) was 5% among the subjects of normal weight, 22% among the overweight, and 60% among the obese.[2]

For the diagnosis of MetS, there are at least three criteria based on five components: waist circumference, blood pressure, blood glucose, triglycerides, and HDL-cholesterol. The National Cholesterol Education Program – Adult Treatment Pannel III (NCEP – ATP III)[3] adopts at least three components for diagnosis of MetS. The IDF[4] considers the abdominal circumference and two more components, and the World Health Organization (WHO)[5] uses the waist/hip ratio, presence of type 2 diabetes mellitus (DM) or insulin resistance, microalbuminuria, hypertension and triglycerides.

Observing the components that classify the individual as having metabolic syndrome, it can be noted that they are all complications that commonly affect obese individuals, which shows that there is a direct link between these two diseases.[6] In general, these two conditions begin to increase in abdominal adipose tissue which is more metabolically active, containing a higher amount of resident macrophages compared to other fat deposits.[7] Abdominal adiposity promotes inflammation and oxidative

stress, which are precursors of various complications involving MetS components, namely insulin resistance, hypertension, and hyperlipidemia (Figure 1).[8,9] Because MetS is associated with cardiovascular complications, the main cause of death worldwide, it is important to understand the factors that are involved in this disorder. Thus, this review aims to present the involvement of oxidative stress in the onset of metabolic syndrome.

Metabolic syndrome and oxidative stress

Inflammation and oxidative stress occur when the energy supply begins to exceed the storage capacity of adipocytes and, as a result, hypertrophy occurs.[10] This hypertrophy leads to a higher release of adipokines as proinflammatory cytokines such as interleukin-1 (IL-1), interleukin-6 (IL-6) and tumor-necrosis factor alpha (TNF-α), resulting in low-grade chronic inflammation, which begins in adipose tissue and eventually reaches the circulation and other organs.[11,12] One of the first consequences of inflammation is insulin resistance, since TNF-α prevents the phosphorylation of insulin receptors, interfering in their cascade action and preventing their functioning.[13] Insulin resistance and type 2 diabetes mellitus are classically characterized by dyslipidemia with hypertriglyceridemia, low HDL-cholesterol and LDL-cholesterol appearance.[14] Insulin resistance decreases insulin function, leading to a change in storage lipids that is a mechanism dependent on this hormone.[15]

Another cause of inflammation is oxidative stress, which can be triggered by adipocytes. When fat mass increases, insufficient irrigation can lead to lack of oxygen and, thus, to cell necrosis. The process of phagocytosis to eliminate these dead cells results in increased inflammatory infiltration and also oxidative stress by liberation of free radicals such as nitric oxide and hydrogen peroxide,[16,17] which may negatively impact components of MetS.[18]

Oxidative stress is classically defined as an event resulting from the magnitude of imbalance between oxidant and antioxidant substances,[19,20] generated in a setting of oxidation-reduction reactions. Since the generation and the action of these substances depend on this oxidation-reduction system, authors now use the term "imbalance of redox system" to refer to the oxidative stress.[21,22] Commonly known as free radicals, oxidants include reactive oxygen and nitrogen species, which perform the oxidation of lipids (lipoxidation) and glucose (glycation), substances found in excess in obesity. Excessive food intake increases the amount of energy and nutrients in the blood stream.[23] Lipoxidation products include malondialdehyde, glyoxal, acrolein, 4-hydroxy-nonenal (HNE), while the

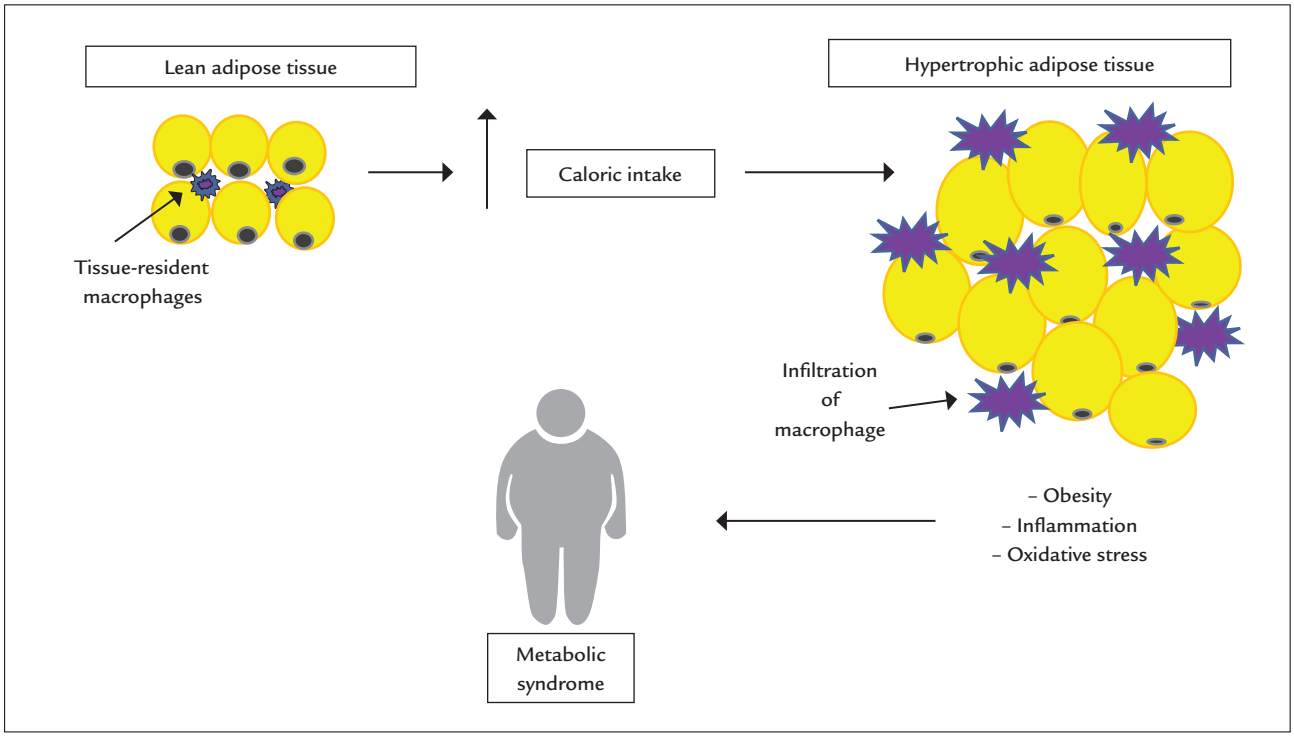

FIGURE 1 Excessive calorie intake leads to hypertrophy of adipose tissue and increased macrophage infiltration, a condition which favors inflammation and oxidative stress situations: a precursor of the metabolic syndrome.

products generated from glycation include glyoxal and methyl glyoxal. These compounds bind to the amino grouping of amino acids, resulting in advanced glycation end-products (AGEs) and advanced lipoxidation end-products (ALEs),[24] which are highly reactive and participate in the development of other components of MetS.

Clinical studies in patients with hypertension showed that systolic and diastolic blood pressure are positively correlated with biomarkers of oxidative stress and negatively correlated with the levels of antioxidants.[25-27] This fact is attributed to endothelial dysfunction caused by oxidative stress and inflammation, producing imbalance of vasoconstrictor and vasodilator products. This is evidenced by an inverse association between factors that trigger vasodilation, plasma levels of malondialdehyde and positive association with antioxidants.[28]

Oxidative stress plays an important role on the pathogenesis of insulin resistance by disrupting the release of adipokines by adipose tissue such as TNF-α and IL-6, which can trigger inflammation, a mechanism already described above.[29-31] Thus, it seems that obesity and MetS are factors associated to inflammation and oxidative stress.

Antioxidant defense

Oxidative stress is controlled by the endogenous antioxidant defense system, which includes antioxidant enzymes such as superoxide dismutase, catalase, glutathione peroxidase, glutathione reductase; and nonenzymatic compounds such as ferritin, transferrin, bilirubin, ceruloplasmin, and even albumin carrier low molecular weight, such as uric acid and lipoic acid.[32] Exogenous antioxidants from fruits and vegetables, including hydrophilic as vitamin C and flavonoids and lipophilic as vitamin E and carotenoids, are also included. Carotenoids are divided into a group of pigments that give yellow and orange colors to plants, animals, and microorganisms. More than 700 carotenoids have been identified; however, lutein, zeaxanthin, cryptoxanthin, alpha-carotene, beta-carotene, and lycopene represent 95% of the carotenoids in human plasma.[33]

Antioxidants are able to trap free radicals generated by cellular metabolism or exogenous sources through the donation of hydrogen atoms of these molecules, breaking the chain reaction, which prevents attack on lipids, amino acids in proteins, double bond of the polyunsaturated fatty acids, and DNA bases, avoiding formation of lesions and loss of cell integrity.[34] Another role of antioxidants is the protection mechanism, which acts in the repair of damage caused by free radicals, a process related to the removal of the DNA molecule of damage and restoration of damaged cell membranes.[35]

The literature reports that a diet rich in fruits, vegetables and grains prevents various diseases, such as cardiovascular diseases and cancer.[36,37] Other intervening factor in antioxidant response and the manifestations of MetS is the association between dietary adequacy and physical exercise.[38] This is due to the exogenous and endogenous antioxidants acting in synergy in combating free radicals.[25] However, it is important to note that this intake needs to be steady and orderly and that the intake of vitamins in supplement form may result in pro-oxidant effects called stress antioxidative.[39]

Biomarkers of oxidative stress

The reactive species are very unstable and have a very short half-life, which makes it a major challenge to perform an accurate assessment of these species. For this reason, methods have been developed for measuring products resulting from the redox markers in biological samples, which are oxidation products of lipids, DNA and proteins.[40] Among the most common are the products of lipid peroxidation because of polyunsaturated fatty acids (such as phospholipids and glycolipids). When these lipids are oxidized, two products classically measured in biological samples, malondialdehyde (MDA) and isoprostan, are formed.[40,41]

MDA is formed by the peroxidation of polyunsaturated fatty acids and can interact with proteins. MDA can be detected by the thiobarbituric acid (TBA) using a colorimetric method based on MDA TBA reaction and form a pink color, so gauging MDA and all species reacting with this acid.[42] The MDA can be specifically measured by high performance liquid chromatography (HPLC). The same reaction occurs between MDA and TBA, but due to the apparatus of the fluorescence detector, only the MDA is identified, making this more specific test.[43]

The isoprostane is a stable product of lipid peroxidation, and can be measured both in the tissues and in biological fluids including urine, plasma, and cerebrospinal fluid. The level of this compound in plasma and urine correlates with the levels of reactive oxygen species and oxidative stress in experimental studies in humans.[44] However, in healthy individuals at risk for obesity and hyperlipidemia their levels are increased, suggesting it as a good marker for cardiovascular risk.[41]

Total antioxidant capacity can be considered a marker of oxidative stress, since it measures the state of antioxidant capacity in biological fluids. This method gives deeper insight into the involvement of oxidative stress in several pathophysiological conditions, but also monitors the effectiveness of antioxidant interventions.[44] In this method the antioxidant capacity of the sample is quanti-

fied by comparing the area under the curve (AUC) on the oxidation kinetics of BODIPY (4,4-difluoro-1,3,5,7,8-pentamethyl-4-bora-3a, 4a-diaza-s-indacene), a fluorescent lipophilic oxidizable compound, radical initiator opposite 2.20 azobis- (2-amidinopropane) dihydrochloride (AAPH) in relation to the oxidation of phosphatidylcholine used as a reference lipid matrix.[45]

Another approach to assess the antioxidant capacity is to measure antioxidants individually. However, as there are many, this would require time and a variety of analytical techniques, instruments, and procedures. In addition, this approach lacks information about the possible synergy and cooperation between the hydrophilic and lipophilic antioxidants.[45]

Protein and DNA molecules are also highly susceptible to modification by changes in redox state.[46] The protein oxidation occurs when proteins of amino acids (proline, arginine, threonine, lysine, histidine and cysteine) bind to glycoxidation and lipoxidation products, forming carbonyl groups. This reaction called carbonylation may be irreversible and lead to changes in their biological function; the detection of these toxic products (carbonyl) can be made by the mass spectrometer.[24]

As for DNA damage, comet assay can be performed, a cell microgel electrophoresis technique, very useful and widely used to assess damage and DNA repair in individual cells. Its basic principle is the lysis of cell membranes, followed by induction of DNA released from the electrophoretic migration on agarose matrix. When viewed under a microscope, the migrated cell takes the apparent form of a comet, with a head, the nuclear region, and a tail, which contains fragments or DNA strands that have migrated towards the anode. The analysis of comets is based on the degree of DNA fragmentation and migration by microelectrophoresis.[47] Measures such as the total length of the "tail" and the DNA density provide indirect information about the state of the sample DNA. To detect oxidative damage, endonuclease III (ENDOIII) and phosphatidylinositol-pyrimidine DNA glycosylase (FPG) are used to repair enzyme thus detecting oxidation bases in the pyrimidine and purine.[48]

Dietary intake, physical activity, oxidative stress, and metabolic syndrome

According to what has been previously described, it is clear that when there is an imbalance within a large supply of nutrients and a low antioxidant intake, obesity carries a picture of oxidative stress promoting metabolic syndrome.[23] Corroborating this fact, the literature indicates that individuals with metabolic syndrome and obesity have a high consumption of fat and sugars derived from processed foods with high sodium content,[49-51] as well as low antioxidant intake.

Diets with high antioxidant content, such as the well-known Mediterranean diet, which consists of olive oil, fruits and vegetables, cereals, nuts, and a small amount of red meat and foods high in sugar, are also ways to manage oxidative stress and inflammation.[52,53] Researchers suggest that individuals with MetS and obesity delayed and attenuated complications, such as insulin resistance, hypertension, and hyperlipidemia, when they had an intervention and began to consume this type of diet. One of the arrangements set out for this improvement was the reduction of oxidative stress and inflammation.[53-57]

Among these studies, Mitjavila et al.[58] observed a decrease of some markers of oxidative stress after one year of dietary intervention. In this same study, subjects with MetS who consumed the Mediterranean diet were compared to a group that consumed a diet with only low levels of fat. It showed that a diet richer in antioxidants resulted in improvement in markers of oxidative stress and decreased DNA damage. This shows the importance of diet quality and the consistency and effectiveness of antioxidants in its composition.

Another factor associated to MetS is reduced daily physical activity in healthy young adults, which leads to negative metabolic consequences such as decreased insulin sensitivity and increased abdominal fat.[59] Therefore, increased physical activity is likely to be the evolutionary favored pathway to prevent the development of insulin resistance during metabolic derangements. The impact of exercise on insulin sensitivity is evident for 24 to 48 hours and disappears within 3 to 5 days, so continuous practice is essential.[2] Besides, exercise increases the production of oxidative stress. However, these increases seem to be necessary in order to allow for an upregulation in endogenous antioxidant defenses, thus providing beneficial effects to the individual engaged in chronic exercise.[60] A combination of resistance and aerobic exercise is the best, but any activity is better than none.

The association between good eating habits and exercise practice is also important. A recent study showed that food adequacy (intake of fruits and vegetables) associated with physical exercise for 20 weeks resulted in higher cardiorespiratory fitness in residents of the city of Botucatu, SP. Moreover, reduction in visceral adiposity (waist circumference) was observed, reducing the prevalence of MetS and mainly increasing significantly glutathione concentration and total antioxidant protection (TAP) of the plasma.[38] Dietary caloric restriction as well as aerobic exercise, an-

aerobic exercise, and resistance training in association with weight loss has been shown to be advantageous in ameliorating oxidative stress and alleviating inflammation in obesity. Studies report that healthy obese patients doing exercise showed decreased lipid peroxidation indicator.[61-63] Thus, it is important to consider strategies that increase the antioxidant defense capacity of the body, since the same part not only of detoxification of free radicals, but are also closely related to modulation of pathophysiologic processes present in the MetS (Figure 2).

Final considerations

This review's approach can highlight the involvement of inflammation, and especially oxidative stress, in the pathogenesis of MetS. Given that obesity may be a key event in the development of this syndrome, treatment strategies are necessary to control and attenuate oxidative stress so that the body does not develop complications leading to MetS.

One of the main factors triggering these two pathologies is food imbalance. In recent decades, there has been an increase in the intake of sugars and fats parallel to a reduction of the consumption of fruit and vegetables; this alone promotes oxidative stress and has been the root cause in the growing epidemic of chronic diseases that affect developed and developing countries. A decrease in food intake coupled with physical activity would be a determining factor in the reduction of oxidative stress. The excess calories from sugar and fat intake, combined with a sedentary lifestyle, cause the body to manage the excess energy that must be metabolized. Other macronutrients undergo oxidation within the mitochondria, promoting an increase in production of free radicals, which has been proposed as a unifying mechanism linking excessive intake of nutrients, insulin resistance, metabolic syndrome, and diabetes. Therefore, what could prevent or delay the onset of MetS would be the maintenance of healthy eating habits, with the inclusion of foods rich in antioxidants and physical activity.

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Conflict of interest

The authors declare no conflict of interest.

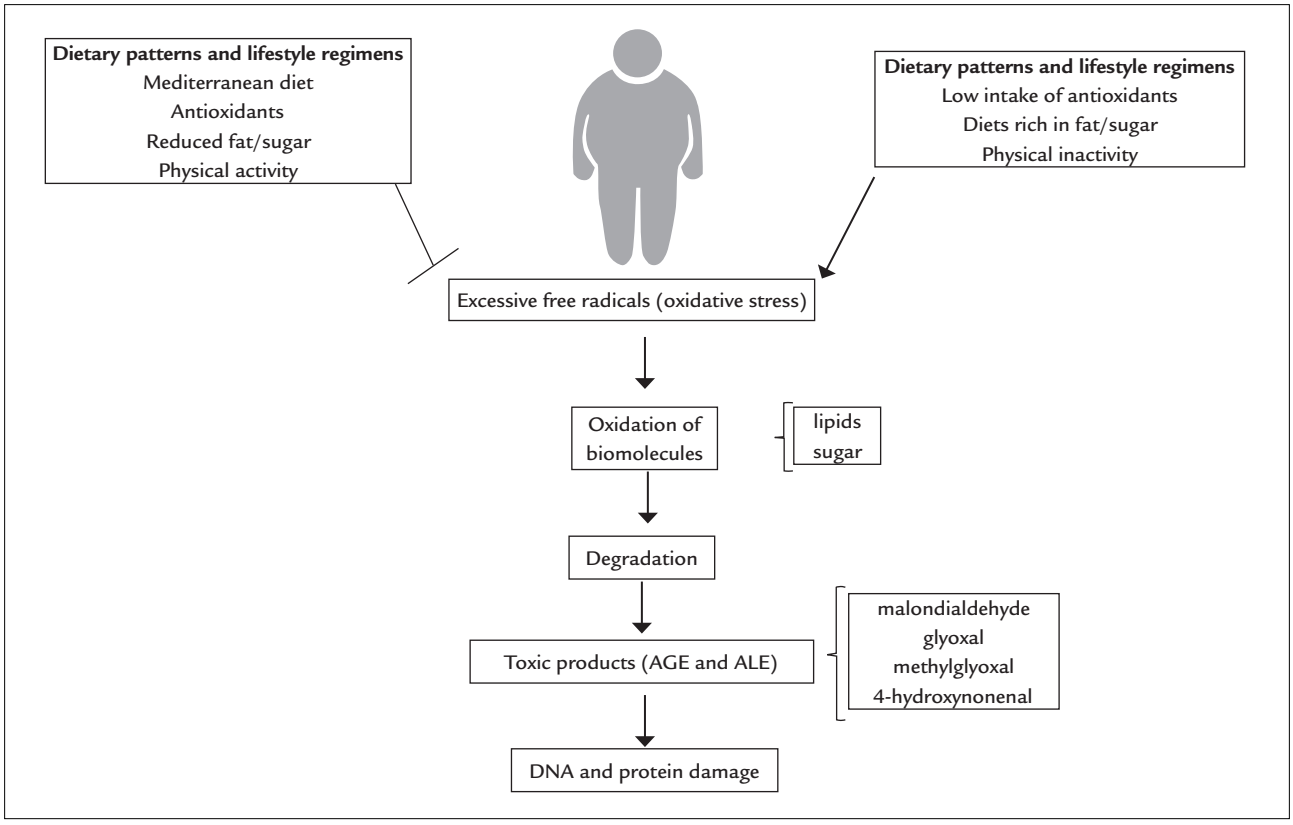

FIGURE 2 Biochemical way of oxidative stress.
AGE: advanced glycation end-products; ALE: advanced lipoxidation end-products.

Resumo

O papel do estresse oxidativo na fisiopatologia da síndrome metabólica

A síndrome metabólica apresenta elevada prevalência na população mundial. De acordo com os componentes que a classificam, nota-se que está intimamente relacionada com a obesidade. Essas duas condições se iniciam com o aumento do tecido adiposo abdominal, o qual é metabolicamente mais ativo, contendo uma quantidade maior de macrófagos residentes em comparação com outros depósitos de gordura. Essa situação favorece a inflamação e o estresse oxidativo, ambos precursores de diversas complicações, mas principalmente as envolvidas na síndrome metabólica, como resistência à insulina, hipertensão arterial e hiperlipidemia. Uma maneira de bloquear os efeitos do estresse oxidativo seria pelo sistema de defesa antioxidante, o qual anula os radicais livres em excesso. É sabido que indivíduos portadores de síndrome metabólica e obesos apresentam um alto consumo de gorduras, açúcares oriundos de alimentos industrializados com alto teor de sódio e uma baixa ingestão de frutas e verduras, apresentando uma condição de estresse oxidativo contínuo. A manutenção de hábitos alimentares saudáveis, com a inclusão de alimentos ricos em antioxidantes, poderia prevenir ou retardar o surgimento da SM.

Palavras-chave: estresse oxidativo, síndrome metabólica, obesidade.

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ERRATUM

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"Metodologias educativas para prevenção de diabetes, hipertensão e obesidade: revisão sistemática"

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