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Cross-sectional study:

- Association between salt taste sensitivity threshold and blood pressure in healthy individuals
- Amount of physical activity necessary for a normal level of high-sensitivity C-reactive protein in ELSA-Brasil
- Use of smartphone-based instant messaging services in medical practice

Systematic review of time series studies:

- What are the perspectives for blood donations and blood component transfusion worldwide?

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Photo: Luciana Silva Rodrigues.

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Nonfatal diseases and quality of life: perspectives in Brazil

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Some diseases kill many people, such as cardiovascular diseases, the most frequent cause of death worldwide. Other diseases do not kill, and they are classified as nonfatal. However, they can compromise the quality of life in such a way that life becomes a huge burden. This burden is quantified in terms of the years lived with disability (YLD). For fatal diseases, the burden is quantified as the YLD plus the years of life lost (YLL). The sum of YLD plus YLL is called disability-adjusted life years (DALYs).

In 1990, coronary heart diseases were the second greatest cause of DALYs. They reached the first position in 2005 and have maintained this position ever since. In 1990, low-back and neck pain were in the seventh position as causes of DALYs, and gradually rose in rank to reach the fourth position in 2013. Depression also rose in rank from the 15th cause of DALYs in 1990 to the 11th position in 2013. In 2013, migraine appeared for the first time as a cause of DALYs, in 25th position.[1]

Considering only YLD, low-back pain occupied the first position in 1990, followed by migraine (2nd), depression (4th), anxiety (6th), other musculoskeletal diseases (7th) and neck pain (8th). Although some changes in position occurred subsequently, all of these were still among the top ten causes of YLD in 2016, as follows:[2] low-back pain (1st), migraine (2nd), depression (5th), other musculoskeletal disorders (6th), neck pain (7th) and anxiety (9th). In the specific case of Brazil in 2016, low-back pain occupied the first position, followed by migraine (2nd), anxiety (3rd), depression (4th), other musculoskeletal disorders (5th) and neck pain (7th).[2]

Data from 2017 described the pattern of conditions associated with DALYs according to sex. In 2017 among women, low-back pain occupied the first position, followed by headaches (2nd), depression (3rd), anxiety (8th), neck pain (9th) and other musculoskeletal conditions (11th); while among men, the ranking was low-back pain (1st), headaches (2nd), depression (5th), other musculoskeletal diseases (10th), neck pain (11th) and anxiety (13th)[3] Thus, despite some changes in the positions of these conditions according to sex, there was no significant difference in relation to low-back pain, headaches and depression. Moreover, although some variables have been measured differently from one study to another, such as migraines in 2013 and 2016, and headaches in 2017, there has been a clear pattern of association between chronic pain and psychiatric disorders over the years in all studies.

What do these data show us? The information that chronic pain is associated with psychiatric disorders is not new. However, how healthcare services are dealing with the association of chronic pain and psychiatric disorders has reached a critical point. It has been reported that in the United States, an epidemic of misuse of opioids is causing 33,000 deaths per year, through both prescribed and illegal use of opioids.[4,5]

In a recent review, Stoicea et al. concluded that with approximately 100 million people suffering from both chronic and acute pain in the United States in 2016, opioids would continue to remain highly prescribed medicines across the US.[5] More than two thirds of overdose episodes in 2016 were opioid-related.[5]

Nonetheless, this is not the first crisis relating to use of opioids over the course of the history of humanity. The ancient Sumerians used a substance medicinally and recreationally around 3300 BC that is thought to have been opium. From there, its use spread across the Middle East and onwards to Greece, India and China. In the 17th century, Portuguese sea merchants profited

from exporting opium to China. The British later took over the trade. When the Chinese government tried to block imports, Britain launched two wars to maintain and expand its massively lucrative drug trafficking. As written elsewhere,[6] "Opium also became used in England and the United States in the form of patent medicines and drinks, which workers consumed to ease the miseries of poverty and parents used to quieten their children". This usage was correlated with the deaths of several children under the age of five years due to narcotic poisoning between 1863 and 1867.[7]

We are not saying that Brazil is at risk of an opioid epidemic. There are many differences in the structure of healthcare services between Brazil and the United States. Brazil has a National Health System with universal access to healthcare, whereas the United States only has Medicare, Medicaid and, more recently, Obamacare. Furthermore, some pain killers that are available in Brazil, such as dipyrone, are prohibited in the United States. Dipyrone is known as "Mexican aspirin" in the United States.

However, it is now important to discuss combined strategies for dealing with chronic pain and psychiatric disorders in Brazil. A trend involving an association between chronic pain and psychiatric disorders has appeared worldwide, and it has been correlated with high YLD. At the end of the second decade of the 21st century, it is more than time to discuss strategies to improve the quality of life of the Brazilian population. One point is obvious: chronic pain and psychiatric disorders walk together.

In the case of headaches, some studies have suggested that these two disorders have risk factors in common.[8-10] The approaches that may be useful in addressing the combination of these two disorders may include introduction of prophylaxis for chronic headaches, treatment of psychiatric disorders and prevention of abuse of pain medication, which is a prevalent cause of headaches.[11] For low-back pain, it may be beneficial to treat pain symptoms through both pharmacological approaches and alternative therapies such as tai chi or acupuncture.[12] Some studies have shown that use of tricyclics has a mild effect regarding improvement of low-back pain.[13] The increasing incidence of obesity and increasing life expectancy in Brazil may complicate the scenario over the coming years through concomitant increases in the incidence of other forms of musculoskeletal pain.

Although some differences according to sex have been observed, the profile of the association of chronic pain and psychiatric disorders is very similar between men and women. This shows that the strategy for addressing the problem can be the same for both sexes.

It is now time to create a strategy for dealing with the association of chronic pain and psychiatric disorders in Brazil, through focusing on diminishing YLD and improving quality of life. Several strategies are possible, beginning with inclusion of pain treatment approaches as part of medical school undergraduate curricula and medical residence training. Most importantly, a solution that is appropriate for Brazil needs to be sought. This should take the structure of the Brazilian healthcare system into consideration and, especially, should be centered on primary care.

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Association between salt taste sensitivity threshold and blood pressure in healthy individuals: a cross-sectional study

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ABSTRACT

BACKGROUND: Hypertension is an important public health problem. Overweight and high salt intake are risk factors for its development.

OBJECTIVE: To evaluate the association between salt taste sensitivity threshold (STST) and blood pressure (BP) in healthy adults.

DESIGN AND SETTING: Cross-sectional study conducted in a private institution.

METHODS: 104 healthy adults (aged 18-59 years) were evaluated. Sociodemographic, clinical and dietary data were collected. Nutritional status and BP were assessed using body mass index (BMI), waist circumference (WC), systolic blood pressure (SBP) and diastolic blood pressure (DBP). STST was assessed using graded saline solutions with sodium chloride concentrations ranging from 0.228 to 58.44 g/l. Identification of salty taste in solutions ≥ 3.652 g/l was used as the cutoff point for high STST.

RESULTS: Participants with high STST presented higher daily average intakes for energy (2017.4 ± 641.5 versus 1650.5 ± 357.7 kcal/day; P = 0.01) and sodium (3070.2 ± 1195.1 versus 2435.2 ± 963.6 mg/day; P = 0.01) and higher BMI (P = 0.008) and WC (P = 0.002). After adjustment for age, sex, sodium and potassium intake, WC and family history of hypertension, the averages for SBP and DBP in subjects with high STST were higher than in those with normal STST (SBP: 138.2 ± 1.7 versus 119.7 ± 0.9 mmHg; P < 0.001; DBP: 81.2 ± 1.9 versus 75.1 ± 1.0 mmHg; P = 0.008).

CONCLUSION: High STST was associated with elevated blood pressure in healthy adults, regardless of other risk factors for hypertension.

INTRODUCTION

Hypertension is a multifactorial clinical condition in which the prevalence differs according to the ethnicity and age group of the population evaluated.[1] The genesis of hypertension involves a number of well-described factors, such as inadequate diet (high amounts of dietary sodium and low potassium intake) and excess adiposity.[2,3] However, the organic response to sodium overload is a mechanism that deserves attention.[4]

Epidemiological studies have suggested that susceptible individuals present elevated blood pressure (BP) through high sodium intake.[5,6] Salt sensitivity is influenced by genetic factors, and not all the population seems to benefit from severe sodium restriction.[7] Meta-analyses and longitudinal studies have suggested that, in comparison with adequate salt intake (4 to 5 g/day), very low sodium intake is associated with increased mortality and cardiovascular events.[8,9] In addition to age and ethnicity, the status of BP levels (normal or elevated/hypertension) seems to be a determinant of the organic response to saline intake.[10]

The salt taste sensitivity threshold (STST) consists of the individual's ability to recognize the taste of sodium. Taste sensitivity decreases according to age and it has been observed that this decrease begins when individuals are around 20 years old.[11] A high STST suggests that the individual is more likely to have excessive salt intake; on the contrary, a normal STST defines that the individual is more likely to have low salt intake. It has been speculated that hypertensive individuals have greater STST than normotensive individuals, which would contribute towards higher sodium intake and, consequently, elevation of BP.[12]

Although the evidence suggests that very low salt intake may contribute negatively to cardiovascular outcomes, reducing sodium intake is still an important public health recommendation as a preventive measure against the incidence of hypertension and as therapy for those who

are already affected by this disease.[13,14] However, the relationship between STST and BP levels has not been completely elucidated in different populations, since decreased sodium consumption seems to exert better effects on BP in hypertensive black and Asian ethnic populations.[10]

OBJECTIVE

The aim of this study was to evaluate the association between salt taste sensitivity, systolic blood pressure (SBP) and diastolic blood pressure (DBP) in clinical practice among healthy adults without hypertension, of predominantly Caucasian ethnicity.

METHODS

A cross-sectional study was carried out between June 2015 and April 2016 among adult students and workers at a university located in southern Brazil, which is a region characterized by European colonization. The sample, which was selected for convenience, consisted of 104 adults of both sexes, aged between 18 and 59 years. Individuals with previous medical diagnoses of hypertension, diabetes mellitus or chronic kidney disease, along with pregnant women, were excluded. The study was approved by the local research ethics committee (CAAE 34989914.9.0000.5310) on September 15, 2014, and the subjects consented to their participation in the study by signing a consent statement.

The participants completed a questionnaire that the researchers had structured, which asked for sociodemographic information (age, sex, self-reported ethnicity, schooling level and income level) and clinical information, including known previous diseases and family history of hypertension (father, mother and grandparents).

To verify the frequency of consumption of food rich in sodium, a specific food frequency questionnaire (FFQ) was used. The instrument asked about 15 foods, and the participants reported the frequency of consumption of each of them, on linear scales marked out as follows: 1 – I never eat this; 2 – I eat this less than once a month; 3 – I eat this one to three times a month; 4 – I eat this once a week; 5 – I eat this two to four times a week; 6 – I eat this once a day; or 7 – I eat this twice or more per day. This questionnaire was developed among low-income Brazilian hypertensive patients and its reliability and validity were tested in the same population.[15]

In addition to the FFQ, a 24-hour food recall (R24h) was used. In this method, the participants reported all types and quantities of food consumed over the 24 hours preceding the collection of information, using domestic measurements. The information obtained from the FFQ and R24h was processed using the DietWin® 2011 nutritional software. The total daily energy intake (TEI, in kcal), macronutrients (carbohydrates, proteins and lipids, as percentages of TEI) and dietary sodium and potassium micronutrients (in mg) were evaluated.

The participants' body mass was measured on a mechanical scale (Welmy®, model 110 CH; Santa Bárbara d'Oeste, SP, Brazil), with a maximum capacity of 150 kg, located on a flat surface and away from walls. This assessment was performed in accordance with the guidelines of the Brazilian Ministry of Health (BMH).[16] These state that the individual needs to be positioned at the center of the calibrated equipment, barefoot, with the minimum of clothes and accessories possible and in an erect posture with feet together and arms extended alongside the body. The subject should remain standing in this position until the weight reading has been made.

Height was measured using an anthropometric ruler with a scale of 2 m, attached to the balance. For this measurement, the individual was kept standing, without shoes, in an erect posture with knees and heels together and arms extended alongside the body, and with the head positioned in accordance with the Frankfurt plan. With the subject in this position, the anthropometer piece at a right angle to the ruler was placed on the top of this individual's head. The measurement was then made with the subject's back, buttocks and head resting against the vertical plane of the anthropometer, in accordance with the BMH recommendations. The participants' nutritional status was determined according to their body mass index (BMI, in kg/m^2).

Waist circumference (WC) was used to evaluate abdominal obesity. It was measured using an inelastic anthropometric measuring tape (Cescorf®; Porto Alegre, Brazil) of 0.1 mm precision and 2 m length. This was placed horizontally at the midpoint between the lower edge of the last rib and the superior iliac crest of each participant. The subjects were placed in a standing position with their abdomen and arms relaxed. The WC criteria adopted for men and women were those established by the Brazilian Society of Cardiology (BSC).[17]

The BP (SBP and DBP) was measured with the participants at rest, in a relaxed sitting position, with uncrossed legs, feet flat on the floor and back resting on the chair. The measurements were made with subject's arm at heart height, free from clothes, with the palm of the hand facing up and elbow slightly flexed, using a properly calibrated device (Omron®, model HEM-710 INT; Kyoto, Japan). Two consecutive readings were made, at a two-minute interval, and the second measurement was used for classification. BP was recorded as a continuous variable, and values greater than or equal to 140/90 mmHg (SBP/DBP) were considered elevated.[17]

To evaluate the STST, nine solutions of sodium chloride (NaCl) were used: 1) 4 mmol/l = 0.228 g/l; 2) 8 mmol/l = 0.456 g/l; 3) 15 mmol/l = 0.913 g/l; 4) 30 mmol/l = 1.826 g/l; 5) 60 mmol/l = 3.652 g/l; 6) 120 mmol/l = 7.305 g/l; 7) 250 mmol/l = 14.610 g/l; 8) 500 mmol/l = 29.220 g/l; and 9) 1,000 mmol/l = 58.440 g/l. The solutions were manipulated in a laboratory of dietetic techniques, using potable water and analytical balance for solute measurements. They

were placed in closed bottles and were kept in a dry environment without light, at room temperature.

The subjects were warned not to smoke, eat or brush their teeth over a period of at least two hours preceding the test. Four drops of the test solution were applied to the tip of the individual's tongue. After 10 seconds without breathing or closing his or her mouth, the subject wrote on a card what the taste felt like. The solutions were offered in increasing concentrations until the individual correctly identified the taste that was felt. After making the correct identification, solutions of decreasing concentrations were then tested, until an error of identification occurred. The concentration immediately higher than this was considered to be the NaCl recognition threshold (STST). Individuals with normal STST were those who identified the salty taste in solutions 1 to 4 (≤ 1.826 g/l of NaCl [30 mmol/l]), while individuals with high STST were those who identified salty taste in solutions 5 to 9 (≥ 3.652 g/l of NaCl [60 mmol/l]). To avoid possible adaptations of the taste sensors, the tests were not done with successive concentrations, but randomly, until the identification. Between the tests, the subjects washed out their mouth with potable water.[12,18]

Data were entered into an Excel® worksheet and statistical analyses were performed in the Statistical Package for the Social Sciences (SPSS®), version 17.0 for Windows. Continuous variables were described as averages and standard deviations, and categorical variables as absolute numbers and frequencies. For comparisons among means, Student's t test was used; and among proportions, Fisher's exact test was used. Correlations were assessed using Pearson's correlation test. Analysis of covariance (ANCOVA) was used to investigate the association between STST and mean blood pressure values, with adjustments according to age, sex, WC, family history of hypertension, sodium intake and potassium intake. The significance level was taken to be 5%.

RESULTS

The average age of the participants was 28.6 ± 7.4 years; 80.8% were female and 98% were self-reportedly of white ethnicity. Among the participants, 51% had completed higher education, and 69.2% reported having a per capita income of up to three Brazilian minimum wages per month. Regarding the family history, 31.7% had no family members with any history of hypertension, 49% had up to three relatives with hypertension and 19.2% had four or more relatives with hypertension.

The average BMI of the participants was 24.1 ± 3.7 kg/m²; among them, 2.9% were considered underweight, 62.5% had normal weight, 28.9% were overweight and 5.8% were obese. The average WC was 76.9 ± 10.2 cm, and 78% of the participants presented normal values. The means for SBP and DBP were, respectively, 123.8 ± 12.3 mmHg and 76.4 ± 9.5 mmHg, and 89.4% of the participants presented normal BP.

Regarding STST, 77.9% presented normal STST and 22.1% presented high STST. **Table 1** shows the characteristics of the sample according to the STST classification. There was no significant difference regarding sociodemographic variables or family history of hypertension in relation to the STST groups. However, participants classified as having higher sensitivity thresholds presented higher mean SBP and DBP, compared with the individuals who were classified as having lower thresholds (P < 0.001).

There was no difference in SBP (P = 0.19) or DBP (P = 0.09) in relation to the presence or absence of a family history of hypertension. Comparing the sexes, it was observed that the mean BMI among men (26.2 ± 3.1 kg/m²) was significantly higher (P = 0.003) than the BMI among women (23.6 ± 3.7 kg/m²). The same relationship was observed regarding WC, for which the mean was significantly higher (P < 0.001) among men (88.1 ± 9.8 cm) than among women (74.3 ± 8.4 cm). Men also presented significantly higher (P = 0.001) SBP (131.9 ± 12.2 mmHg) than women (121.9 ± 11.6 mmHg), whereas there was no significant difference between the sexes regarding DBP (men: 77.9 mmHg; women: 76.1 mmHg; P = 0.43). There was no correlation between age and SBP (P = 0.22). However, the correlation between age and DBP was positive and significant (r = 0.25; P = 0.01). Similarly, the correlations between SBP and BMI (r = 0.39; P < 0.001), SBP and WC (r = 0.47; P < 0.001), DBP and BMI (r = 0.30; P = 0.002) and DBP and WC (r = 0.30; P = 0.002) were positive and significant.

Table 2 presents a comparison of dietary and anthropometric data according to the STST classification. The TEI identified among participants with high STST was significantly higher (P = 0.01) than among subjects with normal STST. No significant difference was observed in relation to the consumption of carbohydrates, proteins and lipids, according to STST levels. The sodium intake of the subjects with high STST was significantly higher (P

Table 1. Characteristics of the sample according to the salt taste sensitivity threshold classification (n = 104)

	Normal STST (n = 81)	High STST (n = 23)	P-value
Age (years)	28.1 ± 7.0	30.7 ± 8.5	0.13*
Sex (%)			0.14‡
Women	84.0	69.6	
Men	16.0	30.4	
Skin color (%)			0.40‡
White	98.8	95.7	
Schooling (%)			0.64‡
Incomplete higher education	50.6	43.5	
Completed higher education	49.4	56.5	
Family history of hypertension (%)	65.4	78.3	0.31‡
Systolic blood pressure (mmHg)	118.9 ± 8.2	141.1 ± 8.1	< 0.001*
Diastolic blood pressure (mmHg)	74.4 ± 8.6	83.61 ± 9.0	< 0.001*

*Student's t test; ‡Fisher's exact test. STST = salt taste sensitivity threshold.

= 0.01) than that of the subjects with normal STST. There was no significant difference in relation to potassium consumption. The BMI of the individuals with high STST was significantly higher than that of the individuals with normal STST (P = 0.008), and this was also found in relation to WC (P = 0.002).

In evaluating the consumption of foods with high sodium content in the entire sample, ham, sausage, pizza and snacks had the highest frequencies, as shown in **Figure 1**. However, no differences were observed in the averages for SBP and DBP in relation to the frequency of consumption of foods with high sodium content. There was also no difference regarding the classification of salt taste sensitivity (normal or high) in relation to the frequency of consumption of these foods (data not shown).

Table 3 shows the SBP and DBP values according to salt taste sensitivity and adjusted for possible confounding factors. The BP values remained higher among individuals with high STST than among those classified as having normal sensitivity, after adjustment for age and sex. The same was observed after adjustments for sodium and potassium intake, WC and family history of hypertension, thus suggesting that an association existed between salt taste sensitivity and BP levels, regardless of other factors relating to BP elevation.

DISCUSSION

In our study, we observed an association between STST and BP levels among young adults of primarily Caucasian origin, regardless of other factors relating to BP elevation, such as age, sex, sodium and potassium intake, WC and family history of hypertension. We also, as expected, identified positive correlations between BP and obesity indicators. Moreover, we observed higher energy and sodium intake among participants with high STST, as well as higher BMI and higher WC. We did not identify any difference in relation to the presence of a family history of hypertension, according to STST status. In addition, there was no difference in SBP and DBP values in relation to the presence or absence of a family history of hypertension.

In Indian adolescents, higher STST and higher BP levels were observed in individuals with a family history of hypertension.[19] It is known that there is a genetic predisposition associated with salt taste perception and that certain populations are genetically more susceptible to development of hypertension.[20,21] Thus, these conditions may be genetically connected, but this association needs to be better explored.[22]

The relationship between excess adiposity and sodium intake has been explored. In our study, we identified that both the BMI and the WC of participants with high STST were significantly higher than in those with normal STST, as also was dietary sodium

Table 2. Dietetic and anthropometric data of the sample according to the salt taste sensitivity threshold classification (n = 104)

	Normal STST (n = 81)	High STST (n = 23)	P-value*
Total daily energy intake (kcal/day)	1650.5 ± 357.7	2017.4 ± 641.5	0.01
Carbohydrate (% from TEI)	50.3 ± 8.1	50.6 ± 7.9	0.86
Protein (% from TEI)	18.7 ± 4.8	17.3 ± 4.5	0.19
Total fat (% from TEI)	30.8 ± 7.6	31.9 ± 7.0	0.53
Dietary sodium (mg/day)	2435.2 ± 963.6	3070.2 ± 1195.1	0.01
Dietary potassium (mg/day)	2116.8 ± 725.5	2219.2 ± 774.5	0.56
Body mass index (kg/m^2)	23.4 ± 2.9	26.6 ± 5.0	0.008
Waist circumference (cm)	74.8 ± 8.1	84.6 ± 13.3	0.002

*Student's t test. TEI = total daily energy intake; STST = salt taste sensitivity threshold.

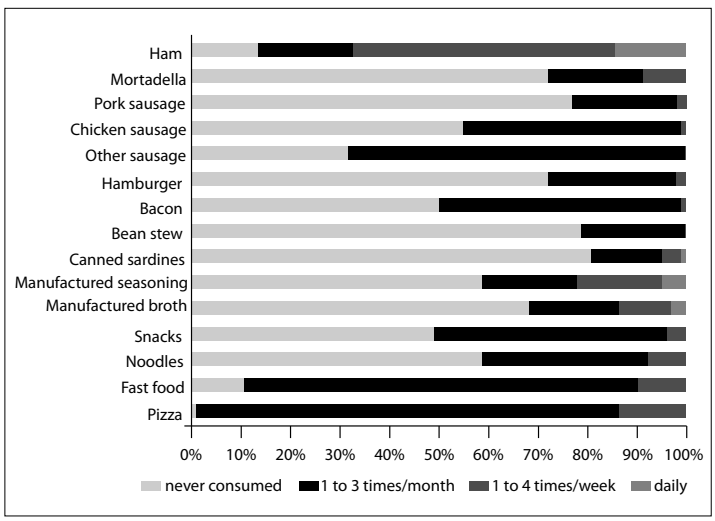

Figure 1. Frequency of consumption of foods with high sodium content in the entire sample (%; n = 104).

Table 3. Adjusted means for arterial blood pressure according to the salt taste sensitivity threshold classification (mean ± standard error; n = 104)

	Systolic blood pressure			Diastolic blood pressure		
	Normal STST (n = 81)	High STST (n = 23)	P-value*	Normal STST (n = 81)	High STST (n = 23)	P-value*
Model 1	119.1 ± 0.9	140.4 ± 1.7	< 0.001	74.55 ± 1.0	83.05 ± 1.8	< 0.001
Model 2	119.2 ± 0.9	140.1 ± 1.7	< 0.001	74.79 ± 1.0	82.52 ± 1.9	< 0.001
Model 3	119.7 ± 0.9	138.5 ± 1.7	< 0.001	74.99 ± 1.0	81.56 ± 1.9	0.004
Model 4	119.8 ± 0.9	138.2 ± 1.7	< 0.001	75.12 ± 1.0	81.15 ± 1.9	0.008

*ANCOVA = analysis of covariance. STST = salt taste sensitivity threshold.

Model 1: Means adjusted for age and sex; Model 2: Means adjusted for model 1 + dietary sodium and potassium intake; Model 3: Means adjusted for model 2 + waist circumference; Model 4: Means adjusted for model 3 + family history of hypertension.

intake. Some authors have identified a positive association between high sodium intake (detected through 24-hour urinary excretion) and changes in body composition in Caucasian populations, with increased body fat and decreased lean mass, regardless of energy intake.[23] Other authors have shown that, for each additional 1 g of ingested salt, the chance of developing obesity is about 26%, regardless of energy intake and ethnicity.[24] Obese individuals have lower STST than do non-obese individuals.[25] However, salt intake appears to be higher among overweight children and adults than among those with normal body mass.[24]

High sodium intake can lead to increased food and energy intake, and it may replace the satiety effect promoted by dietary fats.[26] In our study, we identified higher energy intake among participants with high STST. The mechanisms relating to this association may include elevation of plasma ghrelin concentrations in diets with high sodium content (this hormone acts in the central nervous system [lateral hypothalamus and curved nucleus] to generate the feeling of hunger);[27] and decreased glucagon-like peptide-1 (GLP-1) concentrations in sodium-sensitive individuals when there is an increase in dietary sodium intake (GLP-1 receptors are present in the curved and paraventricular nucleus of the hypothalamus, thus contributing towards reduction of appetite and food intake).[28] In animal models, high sodium intake increases endogenous production of fructose, thereby triggering the processes of leptin resistance and hyperphagia, which result in obesity, insulin resistance and hepatic steatosis among mice.[29]

Differently from Antonello et al., who also evaluated a population in southern Brazil,[30] we observed in our study that there was a significant association between STST and BP levels. Our results are in agreement with other studies conducted among different populations.[31-33] However, there are some controversies regarding the relationship between the salt taste threshold and BP status: in some studies, patients with hypertension have shown a higher recognition threshold for salt,[30,34] while in others it was concluded that there was no difference in STST between people with and without hypertension.[35,36] Studies evaluating potential associations between STST and health outcomes are still scarce and quite heterogeneous regarding the type of design used, the methods used to identify BP levels and sodium intake, the type of population and the number of participants evaluated, which makes it difficult to compare the results.

Among the limitations of this study, we can mention the cross-sectional design, which may have presented the bias of reverse causality; the lack of quantification of urinary sodium and non-performance of 24-hour ambulatory blood pressure monitoring (ABPM); the application of a one-day 24-hour recall, which might not have accurately reflected the dietary habits of the sample (it may underestimate or overestimate food intake, or may characterize flat-slope syndrome); no information about the participants' levels of physical activity was obtained (this is an important factor associated with BP levels); and most of the participants in the study were Caucasians and showed high education levels.

Conversely, we can highlight that our study represented "real life", since ABPM and urinary analysis are not always widely available in clinical practice. Furthermore, we tried to perform analyses that were adjusted for a range of confounding factors that have been correlated with BP changes.

CONCLUSION

An association between STST and BP was observed among the healthy adults participating in this study, regardless of other risk factors for elevation of BP levels, such as age, nutritional status and micronutrient intake. In addition, we identified that the energy and sodium intakes were higher among participants with high STST, and these individuals' BMI and WC were also higher. This result emphasizes the importance of preventive interventions for lifestyle changes, in order to avoid the development of hypertension and other chronic non-communicable diseases.

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Brazilian version of the Self-Estimated Functional Inability because of Pain questionnaire for musculoskeletal injuries relating to dance and sport: translation and cross-cultural adaptation

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ABSTRACT

BACKGROUND: Self-Estimated Functional Inability because of Pain (SEFIP) is a questionnaire specifically designed to measure musculoskeletal pain or discomfort.

OBJECTIVE: To perform translation and cross-cultural adaptation of the SEFIP for dancers (SEFIP-dance), for use in Brazilian Portuguese. In addition, as a secondary objective, we adapted the translated version of SEFIP-dance for use among athletes or exercise practitioners (SEFIP-sport).

DESIGN AND SETTING: Questionnaire translation and cross-cultural adaptation study conducted at a public university.

METHODS: The Brazilian version of the SEFIP-dance questionnaire was developed following the processes of translation (involving two translators with Brazilian Portuguese as their mother tongue and fluency in English), backtranslation (involving two translators with English as their mother tongue and fluency in Brazilian Portuguese), committee review and pre-testing. SEFIP-sport was developed following the processes of content and face validation.

RESULTS: SEFIP-dance was applied to 30 dancers, of mean age 22.38 years (standard deviation [SD] = 3.41), among whom 14 were men (46.66%). The participants understood 100% of the SEFIP-dance items and alternatives. SEFIP-sport was applied to 30 athletes or physical exercise practitioners, of mean age 25.09 years (SD = 8.93), among whom 25 were men (86.33%). The participants understood 100% of the SEFIP-sport items and alternatives.

CONCLUSION: The Brazilian Portuguese versions of SEFIP-dance, translated and cross-culturally adapted for dancers, and SEFIP-sport, adapted for athletes or physical exercise practitioners, were shown to have adequate levels of understanding.

INTRODUCTION

Cross-cultural adaptations of questionnaires in developing countries, such as Brazil, have fostered a major debate involving the fields of economics, health, politics and culture.[1] Today, with the development and dissemination of the Guidelines for the Process of Cross-Cultural Adaptation of Self-Report Measures[2] and of the Consensus-based Standards for the Selection of Health Measurement Instruments (COSMIN),[3] standardization of cross-cultural adaptation relating to culture, language and country is providing positive outcomes within scientific and clinical contexts.

Within healthcare sciences, especially in the field of prevention and rehabilitation of musculoskeletal injuries, it is common to use questionnaires to measure self-reported outcomes, mainly in relation to pain and functional disability.[4-6] Among the questionnaires for screening of musculoskeletal injuries, in addition to instruments that were created by researchers for specific evaluations,[7,8] the Nordic Musculoskeletal Questionnaire (NMQ) stands out through its widespread use for locating musculoskeletal pain in diverse populations.[9-12] However, the NMQ does not have a severity score, and it is not possible to use it to make inferences about functional disability.

Therefore, as a way to fill this gap, the Self-Estimated Functional Inability because of Pain (SEFIP) questionnaire was developed and published in 1999. This is an instrument created based

on the NMQ but with the addition of severity grades relating to functional disability.[13] However, the SEFIP questionnaire was specifically designed to measure musculoskeletal pain or discomfort in dancers. It has nonetheless been used in important studies[14,15] and has already been translated and validated for the Turkish language.[16]

However, there is no validated questionnaire in the Portuguese language to measure pain and screen for musculoskeletal injuries specifically in dancers. Thus, there is justification for such a study. In addition, since the SEFIP questionnaire has a broad and usable structure for individuals who are not involved in dance, we highlight the importance of performing adaptations for its use in other populations, such as athletes[14] and exercise practitioners,[8] who commonly present musculoskeletal pain.

Given the above, the primary objective of this study was to perform the translation and cross-cultural adaptation of the SEFIP questionnaire for dancers (SEFIP-dance), for use in Brazilian Portuguese. In addition, as a secondary objective, we adapted the translated version of SEFIP-dance for use among athletes or exercise practitioners (SEFIP-sport).

METHODS

Study design

This was a cross-sectional study on translation and cross-cultural adaptation of a questionnaire. It was conducted in accordance with the Guidelines for the Process of Cross-cultural Adaptation of Self-Report Measures[2] and the COSMIN.[3] After our institution's research ethics committee had approved the procedures for the study (through opinion number 3.051.824; date: December 3, 2018), the study was conducted at this public university. The recruitment of participants took place in communities around the university by means of verbal disclosure, posters and social media. All participants included in the study validated their participation by signing a free and informed consent statement.

Translation and cross-cultural adaptation of SEFIP-dance

The process of translation and cross-cultural adaptation of SEFIP-dance for Brazilian Portuguese followed the criteria of Beaton et al.[2] and was performed in five stages, as described below.

1) Translation: Two independent translators, comprising one physiotherapist with 10 years of experience in rehabilitation (T1) and one English teacher with 21 years of experience in translation without technical knowledge of the field of healthcare (T2), with Brazilian Portuguese as their mother tongue and fluency in English, translated the original version of SEFIP-dance into Brazilian Portuguese.
2) Synthesis of translations: After discussions and revisions, the two translators, under observation by one of the researchers, synthesized the two independently translated versions of the questionnaire (T1 and T2) and produced a single consensual version of SEFIP-dance (T12).
3) Back-translation: Two independent translators (without technical knowledge of the field of healthcare), both with English as their mother tongue and fluency in Portuguese, translated the Portuguese version of SEFIP-dance back into English, without previous knowledge of the original version of the questionnaire (B1 and B2). These translators were not the same as those in phase 1 (English to Portuguese language translation).
4) Expert committee review: Four rehabilitation experts, together with the four translators involved in the project, reviewed all the translated and back-translated versions for corrections of possible discrepancies, thus reaching the pre-final version of the rehabilitation project. At this stage, the criteria for including rehabilitation experts were as follows: time availability, fluency in both languages, clinical expertise with dancers and interest in collaborating in the study. The pre-final version of SEFIP-dance was agreed among all the committee members.
5) Pre-final test: The pre-final version of SEFIP-dance was applied to 30 dancers with pain in any body region and with Brazilian Portuguese as their mother tongue. The participants read and completed the questionnaire and, at the end of the questionnaire, established that they had understood the pre-final version of SEFIP-dance by selecting check boxes containing "yes" or "no" answers to each question on the questionnaire. If questions were not understood by more than 20% of participants, it was established that they would be reworded and retested among new samples of 30 participants each,[17] until the desired level of understanding was reached, thus arriving at the final version of SEFIP-dance in Brazilian Portuguese.

Sample for adaptation of SEFIP-dance

We used the following eligibility criteria for selecting the adaptation sample: professional dancers and/or dancers who used dance as a recreational activity, with a weekly frequency of at least twice a week; with the ability to read and write in Brazilian Portuguese; without cognitive impairment; and aged 18 years or older.

Adaptation for SEFIP-sport

Initially, two researchers who were directly involved in the SEFIP-dance translation and cross-cultural adaptation process made changes to SEFIP-dance to readdress the questions and items of the questionnaire for athletes and sports practitioners (SEFIP-sport). This first version of SEFIP-sport was then submitted for face and content validation in two phases.[18]

First phase, two physical therapists and two physical education professionals working in the field of sports rehabilitation were consulted with regard to making technical judgments about alterations, inclusions or exclusions of items in the questionnaire and establishing

whether the questionnaire was adequate for measuring musculoskeletal pain-related disability. The criteria for including these experts were the following: time availability, fluency in both languages, clinical and scientific expertise with sports rehabilitation and interest in collaborating in the study.

Second phase, five healthcare professionals were consulted to provide information about possible difficulties in reading the questionnaire and regarding the level of understanding of the items, clarity of response alternatives, presence of typographical errors, size of the letters, length of the questionnaire, time taken for application and overall evaluation of the questionnaire. The criteria for including these experts were the following: time availability, fluency in both languages, clinical expertise with sports rehabilitation and interest in collaborating in the study.

After the face and content validity had been established, the pre-final version of SEFIP-sport was applied to 30 regular practitioners of any sport (who had been doing this for at least 6 months), aged 18 years or older, with Brazilian Portuguese as their mother tongue. The evaluation procedures for the pre-final version of SEFIP-sport followed the same principles as used for SEFIP-dance, thus reaching the final version of SEFIP-sport.

Scoring for SEFIP-dance and SEFIP-sport

Each questionnaire consists of 14 items, each relating to one body part, and it is possible to mark one of five answers for each item, which correspond to scores from 0 to 4. Thus, the total score ranges between 0 and 56 points.[13] The higher the score is, the greater the disability also is. However, to avoid errors in interpreting the magnitude of functional disability through the total score, we suggest that separate analysis should be conducted on each body part, thus resulting in scores ranging from 0 (no pain and disability) to 4 (maximum pain and disability).

Statistical analysis

The data collected during the pre-final test phase were analyzed descriptively through presentation of quantitative variables by means of averages (with standard deviation [SD]) and categorical variables by means of absolute numbers (with percentages). Data processing was performed using the SPSS software, version 17.0 (Chicago, IL, USA).

RESULTS

Translation and cross-cultural adaptation of SEFIP-dance

The translation and back-translation processes are described in **Table 1**. From this process, the pre-final version of SEFIP-dance was defined by the committee of experts. This version was then applied to 30 dancers whose mother tongue was Brazilian Portuguese. The average age of the dancers was 22.38 years (SD = 3.41), and 14 of these participants were men (46.66%). The most common dance

Table 1. Translation, consensus version and backtranslation of the Self-Estimated Functional Inability because of Pain (SEFIP) questionnaire for dancers

SEFIP original	Translation	Consensus version	Backtranslation
Item			
1. Neck	T1: Pescoço T2: Pescoço	T12: Pescoço	B1: Neck B2: Neck
2. Shoulders	T1: Ombros T2: Ombros	T12: Ombros	B1: Shoulders B2: Shoulders
3. Elbows	T1: Cotovelos T2: Cotovelos	T12: Cotovelos	B1: Elbows B2: Elbows
4. Wrists/hands	T1: Punhos/mãos T2: Punhos/mãos	T12: Punhos/mãos	B1: Wrists/hands B2: Wrists/hands
5. Upper back	T1: Parte superior das costas T2: Parte superior das costas	T12: Parte superior das costas	B1: Upper back B2: Upper back
6. Lower back	T1: Parte inferior das costas T2: Parte inferior das costas	T12: Parte inferior das costas	B1: Lower back B2: Lower back
7. Hips	T1: Quadris T2: Quadris	T12: Quadris	B1: Hips B2: Hips
8. Thighs (front)	T1: Coxas (frente) T2: Coxas (parte anterior)	T12: Coxas (frente)	B1: Thighs (front) B2: Thighs (front)
9. Thighs (back)	T1: Coxas (atrás) T2: Coxas (parte posterior)	T12: Coxas (atrás)	B1: Thighs (back) B2: Thighs (back)
10. Knees	T1: Joelhos T2: Joelhos	T12: Joelhos	B1: Knees B2: Knees

Continue...

Table 1. Continuation.

SEFIP original	Translation	Consensus version	Backtranslation
11. Shins	T1: Canelas T2: Pernas (parte anterior)	T12: Pernas (frente)	B1: Legs (front) B2: Legs (front)
12. Calves	T1: Panturrilhas T2: Panturrilhas	T12: Panturrilhas	B1: Calves B2: Calves
13. Ankles/feet	T1: Tornozelo/pés T2: Tornozelos/pés	T12: Tornozelos/pés	B1: Ankles/feet B2: Ankles/feet
14. Toes	T1: Dedos dos pés T2: Dedos (do pé)	T12: Dedos dos pés	B1: Toes B2: Toes
Score			
0. Very well.	T1: Sem dor. T2: Muito bem.	T12: Sem dor.	B1: Without pain. B2: No pain.
1. Some pain but not much problem.	T1: Alguma dor, mas sem muitos problemas. T2: Alguma dor, mas não tem muito problema.	T12: Alguma dor, mas sem muitos problemas.	B1: Some pain, but without many problems. B2: Some pain, but without many problems.
2. Pretty much pain but can handle it.	T1: Bastante dor, mas consigo aguentar. T2: Muita dor, mas eu consigo lidar com isso.	T12: Bastante dor, mas eu consigo suportar.	B1: Quite painful, but bearable. B2: Quite a bit of pain, but I can handle it.
3. Much pain, must avoid some movements.	T1: Muita dor, preciso evitar certos movimentos. T2: Muita dor e devo evitar alguns movimentos.	T12: Muita dor, eu evito certos movimentos.	B1: A lot of pain, I avoid certain movements. B2: A lot of pain, I avoid certain moves
4. Cannot work in the production because of pain.	T1: Não consigo dançar por causa da dor. T2: Não consigo trabalhar na produção por causa da dor.	T12: Não consigo dançar por causa da dor.	B1: I am not able to dance because of the pain. B2: I cannot dance because of the pain.

T1 = Translation 1; T2 = Translation 2; T12 = Consensual synthesis of translations 1 and 2; B1 = Backtranslation 1; B2 = Backtranslation 2.

styles practiced were hip-hop (n = 10; 33.33%), jazz (n = 9; 30%), ballroom (n = 6; 20%) and ballet (n = 5; 16.67%).

The participants understood 100% of the SEFIP-dance items and alternatives, and no changes to the pre-final version were required. The mean total score for SEFIP-dance was 5.63 (SD = 4.66). The "lower back" item was the one most often marked (n = 20; 66.66%), with a mean score of 1.06 (SD = 0.94). The final version of the SEFIP-dance questionnaire in Brazilian Portuguese is presented in **Appendix 1**.

Adaptation for SEFIP-sport

The adaptation for SEFIP-sport was performed based on the version of SEFIP-dance that had been translated and cross-culturally adapted for the Brazilian population. Initially, the following three changes were made to the questionnaire: the alternative with score 4 was changed from *"Não consigo dançar por causa da dor"* ("I cannot dance because of the pain") to *"Não consigo praticar o esporte por causa da dor"* ("I cannot practice the sport because of the pain"); item 13 was changed from *"Tornozelos/pés"* ("Ankles/feet") to *"Tornozelos"* ("Ankles"); and item 14 was changed from *"Dedos dos pés"* ("Toes") to *"Pés"* ("Feet"). In dance, a specific item *"dedos dos pés"* ("toes") is justified due to the common use of this body part, especially in styles such as ballet. However, for various sports, the term "feet" was considered broader and more accurate for SEFIP-sport. The version submitted for face and content validity was approved by 100% of the experts, with no disagreements or suggestions for changes in SEFIP-sport.

Thus, the version approved after the face and content validation was considered to be the pre-final version of SEFIP-sport. Thirty athletes or physical exercise practitioners whose mother tongue was Brazilian Portuguese answered the questionnaire. The mean age of the athletes or exercise practitioners was 25.09 years (standard deviation [SD] = 8.93), and 25 of these participants were male (86.33%). The most common sports practiced were jiu-jitsu (n = 9; 30%), futsal (n = 5; 16.68%), athletics (n = 4; 13.33%), basketball (n = 4; 13.33%), volleyball (n = 4; 13.33%) and karate (n = 4; 13.33%).

The participants understood 100% of the SEFIP-sport items and alternatives, and no changes to the pre-final version were required. The mean total score for SEFIP-sport was 5.07 (SD = 4.25). The item "knee" was the one most often marked (n = 15; 50%), with a mean score of 0.70 (SD = 0.48). The final version of the SEFIP-sport questionnaire in Brazilian Portuguese and English (free translation) are presented in **Appendices 2** and **3**.

DISCUSSION

We performed the translation and cross-cultural adaptation of SEFIP-dance for use in Brazilian Portuguese as the first step in the validation process for this questionnaire. This will allow its future use for investigating musculoskeletal injuries in dancers. In addition, because of the broad characteristics of SEFIP-dance, we were able to change and adjust the translated and adapted version of SEFIP-dance for use among athletes and exercise practitioners (SEFIP-sport).

There are several specific questionnaires in the scientific literature that address complaints relating to certain parts of the body, such as the knee,[19] hip,[20] ankle and foot,[21] shoulder,[22] hand and wrist,[17] cervical spine[23,24] and lumbar spine.[4] However, to assess the presence of musculoskeletal pain or discomfort throughout the body, the NMQ remains the tool most commonly used in research and clinical practice.[8,11,12,25,26]

The NMQ makes it possible to record which part of the subject's body has experienced pain in the last 12 months and 7 days, thus generating a nominal result.[27] In a complementary manner, SEFIP-dance and SEFIP-sport not only allow recording of which body region presented pain at a given moment but also have a disability scale ranging from 0 to 4 points, thereby generating a numerical score for interpretations of pain and disability.[13] Furthermore, it is noteworthy that the two questionnaires adapted here (SEFIP-dance and SEFIP-sport) are easy to understand, since 100% of the sample included in the pre-final evaluation phase understood all the questions.

This study has limitations that need to be considered. SEFIP-dance was translated and cross-culturally adapted based on Beaton et al.[2] and was adapted for practitioners of any sport, based on content and face validity,[18] thus creating a new questionnaire called the SEFIP-sport. Performing only cross-cultural translation and adaptation is common in the scientific literature.[24,28,29] However, these procedures are only the first phase in properly ascertaining the validity of these questionnaires. Therefore, we recommend that further studies should be conducted to verify the psychometric properties of SEFIP-dance in Brazilian Portuguese and SEFIP-sport in Brazilian Portuguese and English.

CONCLUSION

The Brazilian Portuguese versions of SEFIP-dance, translated and cross-culturally adapted for dancers, and SEFIP-sport, adapted for athletes or physical exercise practitioners, were shown to have adequate levels of understanding in the target population for each questionnaire.

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Appendix 1. Brazilian Portuguese version of the Self-Estimated Functional Inability because of Pain questionnaire for dancers (SEFIP-dance).

Self-Estimated Functional Inability because of Pain (Dança)

Nome: _____ Data: _____

Você está sentindo qualquer dor ou desconforto muscular agora? Se sim, indique abaixo até que ponto isso afeta sua dança.
Por favor, marque um quadrado para cada região do corpo.

	Sem dor	Alguma dor, mas sem muitos problemas	Bastante dor, mas eu consigo suportar	Muita dor, eu evito certos movimentos	Não consigo dançar por causa da dor
	(0)	(1)	(2)	(3)	(4)
Pescoço	❑	❑	❑	❑	❑
Ombros	❑	❑	❑	❑	❑
Cotovelos	❑	❑	❑	❑	❑
Punhos/mãos	❑	❑	❑	❑	❑
Parte superior das costas	❑	❑	❑	❑	❑
Parte inferior das costas	❑	❑	❑	❑	❑
Quadris	❑	❑	❑	❑	❑
Coxas (frente)	❑	❑	❑	❑	❑
Coxas (atrás)	❑	❑	❑	❑	❑
Joelhos	❑	❑	❑	❑	❑
Pernas (frente)	❑	❑	❑	❑	❑
Panturrilhas	❑	❑	❑	❑	❑
Tornozelos/pés	❑	❑	❑	❑	❑
Dedos dos pés	❑	❑	❑	❑	❑

Appendix 2. Brazilian Portuguese version of the Self-Estimated Functional Inability because of Pain questionnaire for athletes or exercise practitioners (SEFIP-sport).

Self-Estimated Functional Inability because of Pain (Esporte)

Nome: _____ Data: _____

Você está sentindo qualquer dor ou desconforto muscular agora? Se sim, indique abaixo até que ponto isso afeta sua prática esportiva.
Por favor, marque um quadrado para cada região do corpo.

	Sem dor	Alguma dor, mas sem muitos problemas	Bastante dor, mas eu consigo suportar	Muita dor, eu evito certos movimentos	Não consigo praticar o esporte por causa da dor
	(0)	(1)	(2)	(3)	(4)
Pescoço	❑	❑	❑	❑	❑
Ombros	❑	❑	❑	❑	❑
Cotovelos	❑	❑	❑	❑	❑
Punhos/mãos	❑	❑	❑	❑	❑
Parte superior das costas	❑	❑	❑	❑	❑
Parte inferior das costas	❑	❑	❑	❑	❑
Quadris	❑	❑	❑	❑	❑
Coxas (frente)	❑	❑	❑	❑	❑
Coxas (atrás)	❑	❑	❑	❑	❑
Joelhos	❑	❑	❑	❑	❑
Pernas (frente)	❑	❑	❑	❑	❑
Panturrilhas	❑	❑	❑	❑	❑
Tornozelos	❑	❑	❑	❑	❑
Pés	❑	❑	❑	❑	❑

Appendix 3. English version (free translation) of the Self-Estimated Functional Inability because of Pain questionnaire for athletes or exercise practitioners (SEFIP-sport).

Self-Estimated Functional Inability because of Pain (Sport)

Nome: _____ Data: _____

Are you feeling any muscle pain or discomfort now? If so, indicate below how much it affects your sports practice.
Please check one box for each body region.

	No pain	Some pain, but without many problems	Quite a bit of pain, but I can handle it	A lot of pain, I avoid certain moves	I cannot practice the sport because of the pain
	(0)	(1)	(2)	(3)	(4)
Neck	❑	❑	❑	❑	❑
Shoulders	❑	❑	❑	❑	❑
Elbows	❑	❑	❑	❑	❑
Wrists/hands	❑	❑	❑	❑	❑
Upper back	❑	❑	❑	❑	❑
Lower back	❑	❑	❑	❑	❑
Hips	❑	❑	❑	❑	❑
Thighs (front)	❑	❑	❑	❑	❑
Thighs (back)	❑	❑	❑	❑	❑
Knees	❑	❑	❑	❑	❑
Legs (front)	❑	❑	❑	❑	❑
Calves	❑	❑	❑	❑	❑
Ankles	❑	❑	❑	❑	❑
Feet	❑	❑	❑	❑	❑

Amount of physical activity necessary for a normal level of high-sensitivity C-reactive protein in ELSA-Brasil: a cross-sectional study

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ABSTRACT

BACKGROUND: Studies have shown that physical activity levels can be inversely associated with high-sensitivity C-reactive protein (hs-CRP) levels. However, the amount of physical activity required to maintain normal hs-CRP levels is still a matter for speculation.

OBJECTIVE: To identify the amount of physical activity necessary to discriminate the hs-CRP levels in adults.

DESIGN AND SETTING: Cross-sectional study at six teaching and research institutions.

METHODS: The study sample comprised 10,231 adults aged 35 to 74 years who were participants in the Brazilian Longitudinal Study of Adult Health (ELSA-Brasil). Receiver operating characteristic (ROC) curves were constructed to compare the amount of physical activity in two domains (leisure time and commuting) with hs-CRP levels. The sensitivity and specificity were calculated to identify the best cutoff for physical activity level that would be needed to maintain normal levels of hs-CRP (< 3 mg/l).

RESULTS: The area under the ROC curve was only statistically significant for discriminating normal levels of hs-CRP according to the amount of physical activity when the two study domains were added together. The accumulated physical activity level of 200 minutes/week was the best cutoff for discriminating normal levels of hs-CRP in adults of both sex.

CONCLUSIONS: Physical activity in the leisure-time and commuting domains together, of duration 200 minutes/week, was associated with normal hs-CRP values.

INTRODUCTION

Cardiovascular disease is the leading cause of death and disability worldwide and in Brazil.[1,2] Epidemiological studies conducted over the last 50 years have described risk factors for cardiovascular disease and for coronary heart disease, such as dyslipidemia, hypertension, smoking and diabetes.[3] Moreover, new risk markers such as high-sensitivity C-reactive protein (hs-CRP) levels have been highlighted over recent years as potential predictors of cardiovascular risk.[4,5]

hs-CRP is an acute-phase protein produced in the liver through the primary stimulus of the interleukins IL-1 and IL-6. It has also been assumed that hs-CRP can be produced from the arterial wall. hs-CRP is an extremely sensitive marker for inflammation and tissue damage.[6] Recently, chronic inflammation has been identified as a component in the development and progression of atherosclerosis.[7–9]

The association between regular physical activity and cardiovascular risk factors,[10,11] including hs-CRP,[12,13] has been the subject of evaluations. The MONICA (Multinational Monitoring of trends and determinants in Cardiovascular disease) study in Augsburg, Germany, among men and women aged 35 to 74 years, investigated the association between different domains of physical activity (leisure-time, work, domestic and commuting domains) and markers for inflammation (fibrinogen, hs-CRP and IL-6). An inverse association was found between total physical activity and hs-CRP, even after adjusting for potential confounders.[14,15] Other studies have shown similar results, i.e. indicating significant reduction of hs-CRP levels when moderate exercise is performed.[16,17]

Physical activity is defined as any bodily movement produced by skeletal muscles that results in energy expenditure above resting levels. It may occur within the domains of leisure time, commuting, household or occupational activities.[18] The amount of physical activity that is required

to maintain hs-CRP at normal levels has not yet been established and few studies have provided such information for populations in low and middle-income countries, including Brazil, where chronic non-transmitted diseases comprise the greatest burden on morbidity and mortality rates.[1]

The Brazilian Longitudinal Study of Adult Health (ELSA-Brasil) is a multicenter cohort conducted on adults in Brazil aged 35 to 74. The aim of ELSA-Brasil is to evaluate the complex web of risk factors associated with cardiovascular disease and diabetes, which includes physical activity and hs-CRP.[19]

OBJECTIVE

The aim of the present study was to identify the amount of physical activity that was required to discriminate normal hs-CRP levels among men and women participating in ELSA-Brasil.

METHODS

Study population

In the Brazilian Longitudinal Study of Adult Health (ELSA-Brasil), 15,105 participants of both sexes aged between 35 to 74 years were recruited in six state capitals in Brazil (Rio Grande do Sul, São Paulo, Rio de Janeiro, Belo Horizonte, Vitória and Salvador).[19,20] In this analysis, we selected 10,231 participants from the baseline (2008-2010), through exclusion of those who reported that they had previously had cardiovascular disease, were using statins, were diabetic, had not undergone hs-CRP determination, had not given responses to the physical activity questionnaire and presented hs-CRP levels above 10 mg/l, since this level is more suggestive of acute infection.[21]

Ethical considerations

ELSA-Brasil was approved by the National Commission for Research Ethics and by the research ethics committees of all the six centers involved in these investigations: Universidade Federal da Bahia (under the registration number 027-06); Hospital Universitário da Universidade de São Paulo (669/06); Fundação Oswaldo Cruz (343/06); Universidade Federal de Minas Gerais (186/06); Hospital de Clínicas de Porto Alegre (06-194); and Universidade Federal do Espírito Santo (041/06). All participants signed a free and informed consent form and were guaranteed secrecy and confidentiality of information.

Data collection

Data were collected by a team of interviewers and measurement technicians who had received training to implement the study protocol. This team was supervised by qualified professionals. Interviews were conducted face-to-face, and anthropometric measurements and blood collection were performed on the same day as the interview.[22]

Sociodemographic variables were determined: sex, educational level (university degree, complete secondary school, completed elementary school or incomplete elementary school), self-identified skin color/race category (white, brown, black, Asian or indigenous), age and functional status (active or retired).

To evaluate nutritional status, the body mass index (BMI) was used, determined as the ratio between weight and height squared. Weight and height were measured by trained personnel in the six centers.

Physical activity was measured using the International Physical Activity Questionnaire (IPAQ), long version, in the domains of physical activity during leisure time (LTPA) and commuting physical activity (CPA). This instrument had previously been validated for use in Brazil. It consists of questions concerning the frequency, duration and intensity of physical activity: moderate-intensity walking within LTPA, i.e. needing some physical effort that makes breathing a little stronger than normal; vigorous-intensity walking within LTPA, i.e. needing great physical effort that makes breathing much stronger than normal; and walking and cycling within CPA.[23] The amount of physical activity in its different domains was reported in minutes/week and was ascertained by multiplying the weekly rate by the duration of each of the activities. Physical activity was considered to be an activity undertaken for at least 10 minutes/week.

Laboratory procedures

Blood samples were collected in the six research centers after a mean period of 12 hours of overnight fasting. The samples were stored in dry tubes. To ensure the quality and standardization of results, the processing and analysis of the material were performed in a central ELSA-Brasil laboratory. The hs-CRP levels were measured by means of the quantitative nephelometry method (BN II, Siemens), and the results were expressed as milligrams per liter (mg/l). The hs-CRP measurements were dichotomized regarding cardiovascular risk, in accordance with the recommendations from the Centers for Disease Control and Prevention (CDC) and the American Heart Association (AHA), as high risk (≥ 3 mg/l) and medium/low risk (< 3 mg/l).[24]

Data analysis

Central trend measurements, frequencies and 95% confidence intervals were calculated for the variables of interest. The predictive power and cutoff points of the LTPA, CPA and combined LTPA + CPA domains for normal levels of hs-CRP were identified from receiver operating characteristic (ROC) curves, which are commonly used to determine cutoff points in diagnostic tests or screening.[25]

Initially, the total area under the ROC curve relating to the amount of physical activity performed (in minutes/week) was identified according to the categories of moderate and vigorous

walking in the leisure-time domain (LTPA), walking and cycling in the commuting domain (CPA) and these activities in the summed LTPA + CPA, considering normal levels of hs-CRP. The larger the area under the ROC curve was, the greater the discriminatory power of physical activity for normal levels of hs-CRP also was. The 95% confidence interval was used to determine whether the discriminatory capacity of the physical activity patterns in the domains investigated was or was not due to chance, and whether this should or should not be included in the range of values ≤ 0.50.

To test the differences between the areas under the receiver operating characteristic (ROC) curve, the chi-square test was used, considering a 5% significance level. Sensitivity and specificity were calculated, along with cutoffs for the amounts of physical activity needed to maintain normal levels of CRP, using the Youden index to indicate the optimal cutoff and maximize the overall effectiveness rating for diagnostic purposes.[26] The data were analyzed using the Stata statistical software, version 12 (Stata Corporation, College Station, United States).

RESULTS

Out of the 10,231 participants selected for this study, 55.9% were women. The average age was about 50.1 years (standard deviation, SD = 0.1) for men and women together. A higher proportion of the men had educational levels of incomplete and completed elementary school, compared with the women. On the other hand, more than half of the study population had a university degree (55.7%). Regarding employment status, a higher proportion of the women among the employees were retired at the time of the interview (16.6%). It was noteworthy that the frequency of overweight men was higher (44.7%), but more than half of the study population (57.3%) had BMI levels compatible with overweight or obesity. About a quarter of the participants presented high hs-CRP levels (> 3 mg/l) during the investigation period (**Table 1**).

In assessing the amount of physical activity during leisure time that was practiced for at least 10 minutes per week, 42.9% of the study participants of both sexes were found to be inactive.

Table 1. Distribution of participants according to selected characteristics and sex. ELSA-Brasil, 2008-2010

Characteristics	Total			Women			Men		
	n	%	95% CI	n	%	95% CI	n	%	95% CI
Age									
Mean (SD)		50.1 (0.1)			50.2 (0.1)			50.0 (0.1)	
35-44	2,866	28.0	(27.1-28.9)	1,552	27.1	(26.0-28.3)	1,314	29.1	(27.8-30.5)
45-54	4,325	42.3	(41.3-43.2)	2,433	42.6	(41.3-43.8)	1,892	41.9	(40.5-43.3)
55-64	2,374	23.2	(22.4-24.0)	1,388	24.3	(23.2-25.4)	986	21.9	(20.6-23.0)
65-74	666	6.5	(6.0-7.0)	344	6.0	(5.4-6.7)	322	7.1	(6.4-7.9)
Educational level									
Incomplete elementary	449	4.4	(4.0-4.8)	166	2.9	(2.5-3.4)	283	6.3	(5.6-7.0)
Completed elementary	580	5.7	(5.2-6.1)	244	4.3	(3.8-4.8)	336	7.4	(6.7-8.2)
Completed high school	3,500	34.2	(33.3-35.1)	1,981	34.6	(33.4-35.9)	1,519	33.7	(32.3-35.0)
University degree	5,702	55.7	(54.8-56.7)	3,326	58.2	(56.9-59.5)	2,376	52.6	(51.2-54.1)
Color/race									
Black	1,487	14.7	(14.0-15.4)	909	16.1	(15.1-17.0)	578	12.9	(12.0-14.0)
Brown	2,900	28.7	(27.8-29.5)	1,524	26.9	(25.8-28.1)	1,376	30.9	(29.5-32.2)
White	5,407	53.4	(52.4-54.4)	3,035	53.6	(52.3-54.9)	2,372	53.2	(51.7-54.7)
Asian	232	2.3	(2.0-2.6)	155	2.7	(2.3-3.2)	77	1.7	(1.4-2.1)
Indigenous	94	0.9	(0.7-1.1)	38	0.7	(0.5-0.9)	56	1.3	(0.9-1.6)
Functional status									
Active	8,811	86.1	(85.4-86.8)	4,769	83.4	(82.4-84.4)	4,042	89.5	(88.6-90.4)
Retired	1,420	13.9	(13.2-14.6)	948	16.6	(15.6-17.6)	472	10.5	(9.6-11.4)
Nutritional status									
Underweight	117	1.1	(0.9-1.4)	64	1.1	(0.8-1.4)	53	1.2	(0.8-1.5)
Normal	4,251	41.6	(40.6-42.5)	2,530	44.3	(43.0-45.6)	1,721	38.1	(36.7-40.0)
Overweight	4,053	39.6	(38.7-40.6)	2,037	35.6	(34.4-36.9)	2,016	44.7	(43.2-46.1)
Obese	1,807	17.7	(16.9-18.4)	1,085	19.0	(18.0-20.0)	722	16.0	(15.0-17.1)
C-reactive protein									
Median (25%-75%)		1.29 (0.66-2.76)			1.42 (0.71-3.15)			1.15 (0.62-2.33)	
< 3 mg/l	7,897	77.2	(76.4-78.0)	4,191	73.3	(72.1-74.4)	3,706	82.1	(80.9-83.2)
≥ 3 mg/l	2,334	22.8	(22.0-23.6)	1,526	26.7	(25.5-27.9)	808	17.9	(16.8-19.0)

ELSA-Brasil = Brazilian Longitudinal Study of Adult Health; SD = standard deviation; CI = confidence interval.
The combined total differs due to loss of information on some variables.

Regarding commuting, it was found that 72.7% were walking and 4.7% were using a bicycle to move for at least 10 minutes per week. Considering the time spent in LTPA and CPA together, 12.6% of respondents reported that they were not doing at least 10 minutes of physical activity during the week (**Table 2**).

Areas under the ROC curve that were larger than 0.50 and statistically significant for discriminating normal hs-CRP levels were found in the LTPA domain, independent of intensity, for both sexes. The analysis on physical activity relating to the manner of commuting (walking and cycling) showed that none of the areas under the curve was statistically significant for discriminating normal levels of hs-CRP. However, the sum of physical activity in the two domains showed an area under the ROC curve that was statistically significant for discriminating normal hs-CRP levels in both men and women (**Table 3**).

Evaluation according to gender, functional status, BMI and age showed that the areas under the ROC curve relating to the sum of the amount of physical activity in the two domains as a discriminator of normal hs-CRP levels were not statistically significant (**Figure 1**). The cutoff for the amount of physical activity that best discriminated normal levels of hs-CRP, with sensitivity of 52% and specificity of 55%, was 200 minutes/week for both sexes, when practices during leisure time and commuting were assessed together (**Figure 2**).

DISCUSSION

This study demonstrated the discriminatory power of the sum of physical activity in the two domains studied (LTPA + CPA) for normal levels of hs-CRP in adults. It also found that the level of the cutoff for physical activity in minutes per week showed better balance between sensitivity and specificity for discriminating normal levels of hs-CRP in both sexes.

It was observed that a large proportion of this population was not performing at least 10 minutes of moderate or vigorous LTPA, even though the recommended minimum is 150 minutes per week.[27] The use of bicycles for commuting (4.7%) was lower than in other Brazilian cities and also lower than in European countries such as the Netherlands.[28,29] These differences can be explained by various individual, environmental and psychosocial factors, as well as cultural issues.[28,30]

It was striking that the LTPA domain presented a significant area under the ROC curve (> 0.50) at all intensities (moderate and vigorous physical activity of walking), which translates as discriminatory power for normal levels of hs-CRP. However, other studies

Table 2. Distribution of participants according to physical activity for at least 10 minutes/week, domain, intensity and sex. ELSA-Brasil, 2008-2010

Characteristics	Total			Women			Men		
	n	%	95% CI	n	%	95% CI	n	%	95% CI
Leisure-time domain									
Walking									
Yes	3,983	38.9	(38.0-39.9)	2,071	36.2	(35.0-37.5)	1,912	42.4	(40.9-43.8)
No	6,248	61.1	(60.1-61.0)	3,646	63.8	(62.5-65.0)	2,602	57.6	(56.2-59.1)
Moderate									
Yes	2,333	22.8	(22.0-23.6)	1,200	21.0	(19.9-22.1)	1,133	25.1	(23.8-26.4)
No	7,898	77.2	(76.4-78.0)	4,517	79.0	(77.9-80.0)	3,381	74.9	(73.6-76.2)
Vigorous									
Yes	2,504	24.5	(23.6-25.3)	1,055	18.5	(17.4-19.5)	1,449	32.1	(30.7-33.5)
No	7,727	75.5	(74.7-76.3)	4,662	81.5	(80.5-82.5)	3,065	67.9	(66.5-69.3)
Total									
Yes	5,841	57.1	(56.1-58.5)	2,977	52.1	(50.8-53.4)	2,864	63.5	(62.0-64.8)
No	4,390	42.9	(41.9-43.9)	2,740	47.9	(46.3-49.2)	1,650	36.5	(35.1-38.0)
Commuting domain									
Walking									
Yes	7,443	72.7	(71.9-73.6)	4,085	71.4	(70.6-72.6)	3,358	74.4	(73.1-75.7)
No	2,788	27.3	(26.4-28.1)	1,632	28.6	(27.4-29.7)	1,156	25.6	(24.3-26.9)
Cycling									
Yes	478	4.7	(4.3-5.1)	74	1.3	(1.0-1.6)	404	9.0	(8.1-9.8)
No	9,753	95.3	(94.9-95.7)	5,643	98.7	(98.4-99.0)	4,110	91.0	(90.2-91.9)
Total									
Yes	7,542	73.7	(72.8-74.6)	4,105	71.8	(70.6-73.0)	3,437	76.1	(74.9-77.4)
No	2,689	26.3	(25.4-27.1)	1,612	28.2	(27.0-29.9)	1,077	23.9	(22.6-25.1)
Leisure-time + commuting domain									
Yes	8,939	87.4	(86.7-88.0)	4,901	85.7	(84.8-86.6)	4,038	89.5	(88.5-90.3)
No	1,292	12.6	(12.0-13.3)	816	14.3	(13.4-15.2)	476	10.5	(9.7-11.5)

ELSA-Brasil = Brazilian Longitudinal Study of Adult Health; CI = confidence interval.

with similar methodology, but in which the outcomes were presence of diabetes and visceral fat did not enable recognition of any significant discriminatory power at all intensities of LTPA.[31,32] The domain of CPA alone was unable to discriminate normal levels of hs-CRP.

The discussion about the amount of physical activity needed for promotion of good health conditions is not new. In previous studies, results with a dose-response relationship between the level of physical activity and the chance of health problems

Table 3. Areas under the ROC curve and 95% CI of the amount of physical activity as a discriminator for normal hs-CRP levels in adults. ELSA-Brasil, 2008-2010

Domains	Women	Men	Total
PA during leisure time			
Walking	0.53 (0.52-0.55)*	0.53 (0.51-0.55)*	0.54 (0.52-0.55)*
Moderate	0.52 (0.51-0.53)*	0.53 (0.52-0.55)*	0.53 (0.52-0.53)*
Vigorous	0.53 (0.52-0.54)*	0.53 (0.51-0.54)*	0.54 (0.53-0.55)*
Total	0.54 (0.53-0.56)*	0.54 (0.52-0.56)*	0.55 (0.54-0.56)*
PA during commuting			
Walking	0.50 (0.48-0.51)	0.51 (0.49-0.53)	0.50 (0.49-0.52)
Cycling	0.50 (0.50-0.51)	0.50 (0.49-0.51)	0.51 (0.50-0.51)
Total	0.50 (0.49-0.52)	0.51 (0.49-0.52)	0.51 (0.50-0.52)
Sum of PA			
Leisure time + commuting	0.52 (0.51-0.53)*	0.52 (0.51-0.53)*	0.52 (0.51-0.53)*

ROC = receiver operating characteristic; CI = confidence interval; hs-CRP = high-sensitivity C-reactive protein; ELSA-Brasil = Brazilian Longitudinal Study of Adult Health; PA = physical activity.

*Area under the ROC curve showing discriminatory power for normal levels of hs-CRP (CI ≥ 0.50).

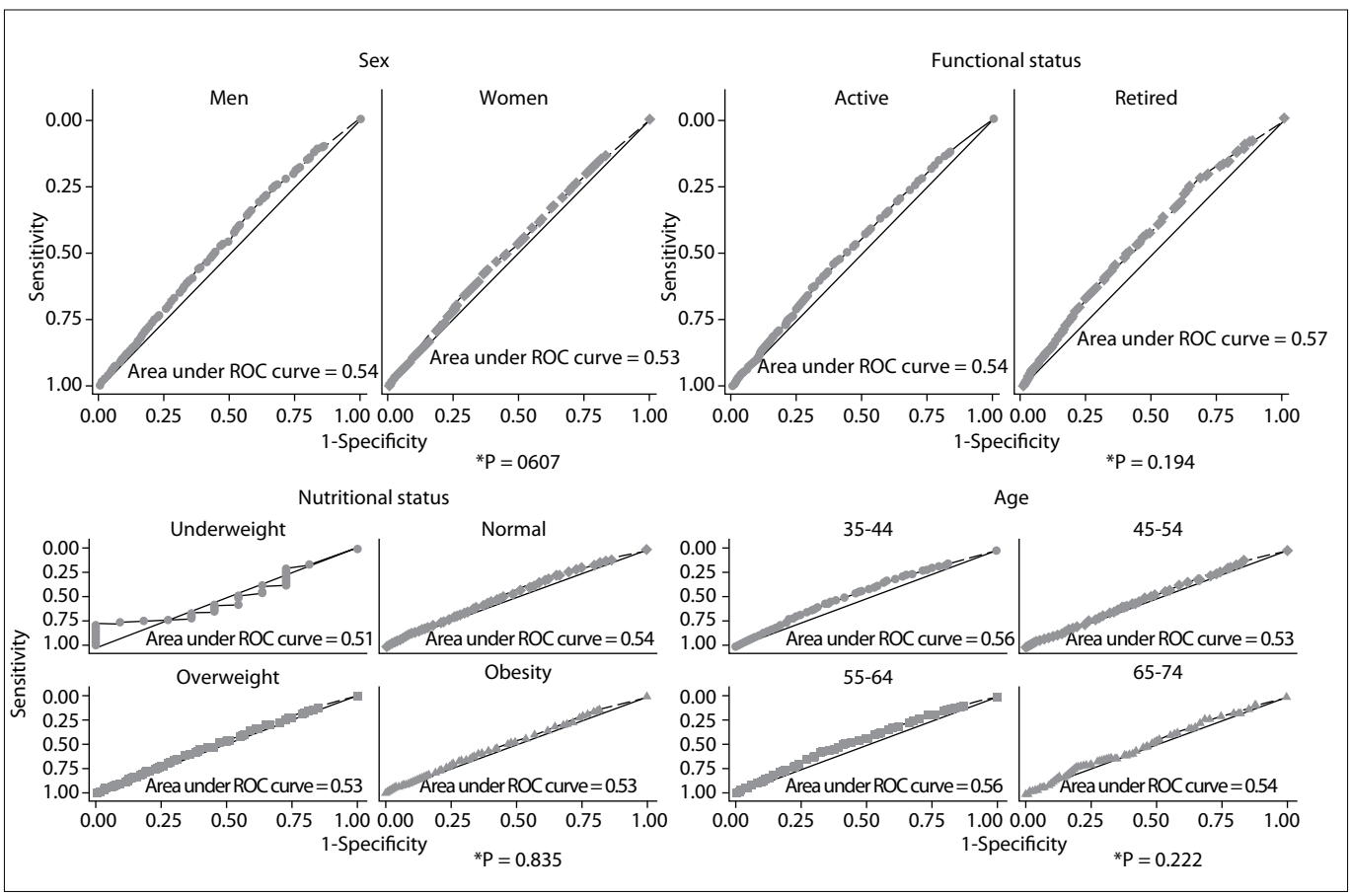
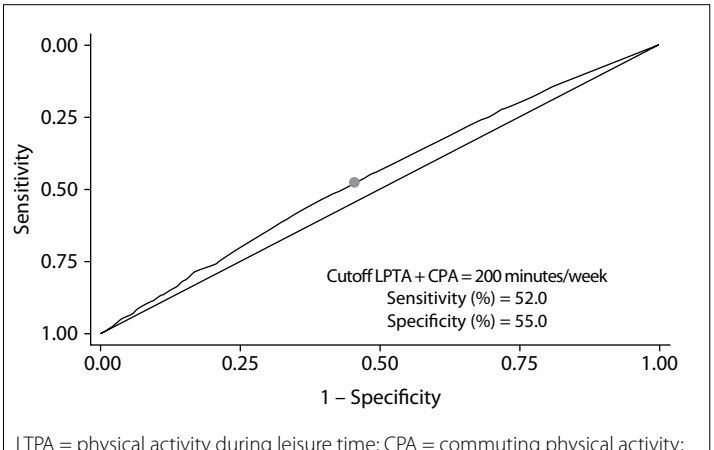


Figure 1. Comparison of the areas under the receiver operating characteristic (ROC) curve of the sum of the amount of physical activity in the two domains studied, for discriminating normal levels of high-sensitivity C-reactive protein (hs-CRP), stratified according to sex, functional status, age and body mass index. Brazilian Longitudinal Study of Adult Health (ELSA-Brasil), 2008-2010.

had also been observed, especially with regard to metabolic and cardiovascular problems.[33] Studies have shown that less than 150 minutes of physical activity per week is not enough to provide significant benefits regarding protection against some types of health problems.[27,34] Nonetheless, contrary to the 2008 recommendations, around 31% of the population of the present study was physically inactive.[35,36]

The benefits that physical activity can provide in reducing hs-CRP levels have been demonstrated in the literature, but few studies have attempted to identify the discriminatory power of different durations and intensities of physical activity, to keep hs-CRP at normal levels.[16,17] In a survey conducted in Finland using a population-based sample, inverse associations between the level of physical activity during leisure time and hs-CRP levels was found for both sexes and between the level of physical activity and hs-CRP levels during commuting for women, even after adjusting for confounding factors.[37]

It was hypothesized in a previous study that one potential route towards reducing hs-CRP concentrations in physically active subjects might be through interleukin levels, especially IL-6 and tumor necrosis factor (TNF-α), but this was not the main subject of that study.[38] In the present study, it was observed that the amount of physical activity that discriminated normal levels of hs-CRP when evaluated according to functional status, age, obesity and sex did not show any statistically significant difference in area under the ROC curve. It was therefore decided to identify a single cutoff that would cover all the categories evaluated. It was found that only the sum of accumulated physical activity in the two domains studied (LTPA + CPA) had the power to determine the cutoff: 200 minutes per week of physical activity for both sexes. When these domains were analyzed separately, the results regarding the area under the ROC curve were not significant, or the sensitivity and specificity values were not adequate.



LTPA = physical activity during leisure time; CPA = commuting physical activity; min = minutes.

Figure 2. Cutoff point, sensitivity and specificity for the sum of physical activity in the two domains studied (LTPA + CPA), for discriminating normal hs-CRP levels. Brazilian Longitudinal Study of Adult Health (ELSA-Brasil), 2008-2010.

Similar results were found in another study conducted in Brazil, in which the outcome studied was diabetes. It was found that 215 minutes of physical activity per week, accumulated over the four domains for women, and 185 minutes of physical activity per week, accumulated over the four domains for men, were the best cutoffs for discriminating the absence of diabetes.[32]

In ELSA-Brasil, it was decided as a methodological strategy only to use the domains of physical activity during leisure time and commuting. However, the International Physical Activity Questionnaire (IPAQ) also includes the domains of physical activity at work and domestic physical activity. The decision not to use these last two domains was made on the basis of studies in the literature, in which a trend of overestimation in the results from these studies in Latin America has been described.[39]

One possible limitation of the present study relates to its use of an indirect measurement for evaluation of physical activity. It may be assumed, for example, that people who are more physically active tend to underestimate the time spent performing their activities, while those who are less active are more prone to overestimation.[40] It should also be noted that questionnaire-type instruments are more susceptible to recall bias. However, such instruments are used in around two-thirds of countries worldwide, thus enabling benchmarking between them.[35]

It was found in the present study that the sensitivity and specificity values were considered low with regard to diagnostic testing or screening for diseases. However, in considering physical activity as a discriminator, it should not be interpreted as a diagnostic test, but as a form of behavior that presents components and social, biological, psychosocial, cultural and economic factors that make it much more complex. In addition, regular physical activity can bring benefits regarding disease prevention and health promotion.[41] Similar sensitivity and specificity values were also found in research on physical activity in which the study design was similar but the outcomes were the presence of visceral fat and diabetes. In these studies, the sensitivity ranged from 58% to 68% and the specificity ranged from 52% to 68%.[31,32]

It can be concluded that accumulated physical activity practices in the leisure time and commuting domains can contribute towards maintaining normal hs-CRP levels. The results suggest that individuals should practice 200 minutes/week of physical activity to maintain hs-CRP at appropriate levels. These results underpin the recommendations of public health policies and programs that aim towards preventing non-communicable diseases, with community interventions, and especially those that stimulate increased physical activity levels in the adult population.

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Academic performance and use of psychoactive drugs among healthcare students at a university in southern Brazil: cross-sectional study

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ABSTRACT

BACKGROUND: People have been using psychoactive substances for a long time. Over the last few years, this practice has spread among university students, who use these substances to improve their academic performance, relieve stress and increase concentration and memory.

OBJECTIVES: To estimate the use of psychoactive drugs among healthcare students at a higher education institution in the city of Passo Fundo (RS), Brazil, and to ascertain the associated demographic and lifestyle factors.

DESIGN AND SETTING: Cross-sectional study in a higher education institution.

METHODS: We included 287 undergraduate medicine and dentistry students in this study. They answered a self-administered questionnaire regarding sociodemographic, lifestyle and health variables. The statistical analysis used univariate and bivariate analyses with Pearson's chi-square test (P-value < 0.05). Multivariate analyses were used to estimate odds ratios (OR) and their respective 95% confidence intervals. The SPSS software, version 20.0, was used.

RESULTS: The prevalence of use of psychoactive substances among the students was 24.7%. Among these students, high frequencies of psychoactive drugs had been prescribed by physicians (95.8%) and for the purpose of relaxation or stress relief (73.2%). Women, medical students (compared with dental students) and participants with lower academic performance were more likely to use psychoactive drugs. After the multivariate adjustment, the "course" and "academic performance" remained associated with use of psychoactive drugs.

CONCLUSION: There was high prevalence of psychoactive drug use among the students at the higher education institution investigated. Some variables (female sex, medical students and low academic performance) were associated with the outcome.

INTRODUCTION

Throughout history, humans have resorted to the use of psychoactive substances for various purposes. Psychoactive drugs work on the brain, modifying its operation and changing mood, behavior and consciousness, which may lead to a state of dependence. Among these substances are licit drugs (alcohol and tobacco), illicit drugs (marijuana, cocaine, crack and others) and psychoactive drugs (tranquilizers, sedatives and strong analgesics).[1]

Generations of students have used brain stimulants, food supplements, drugs subject to medical prescription, amphetamines and energy drinks in order to enhance their mental faculties, namely memory, concentration, reasoning and language, which are inherent to studying and academic development.[2-4]

The main substances used for this purpose are caffeine, methylphenidate (MPH), modafinil, piracetam, energy drinks and amphetamines. Although the specific action mechanisms may vary, psychostimulants usually work directly or indirectly through dopamine[5,6], motivation, attention and excitement.[7]

MPH (also known as Ritalin™) and dextroamphetamine (d-AMP) are the most commonly used non-medical substances, although other amphetamine formulations have also been used. However, these substances, which belong to the class of amphetamines, present significant risk of dependence.[8] Among students using methylphenidate, most of them have used it in highly stressful periods of academic studies.[9] Thus, studies have estimated that 5% to 35% of university students use these drugs to improve their academic performance, stimulate cognitive function and decrease physical and mental fatigue.[2,10]

Hence, the increasing use of psychoactive drugs for non-therapeutic purposes creates the need to inform students and raise their awareness about the real risks of over-the-counter psychoactive drugs and their harmful effects on health, such as increased blood pressure, arrhythmias, headaches, overdose and depression.[2,10,11] Therefore, studies providing data on the use of psychoactive substances among healthcare students become relevant. Understanding and reflecting on the issues raised by this topic may allow the creation of effective intervention strategies.

OBJECTIVE

The aim of the present study was to estimate the use of psychoactive drugs among healthcare students at a higher education institution located in the city of Passo Fundo (RS), Brazil, and to ascertain the associated demographic and lifestyle factors.

METHODS

Ethical considerations

This study was approved by the institution's ethics committee, under investigation no. 2.014.448 and CAAE 66373317.6.0000.5319 (April 12, 2017). The voluntary nature of the participation and the confidential nature of the information were explained to the students. All participants signed a free and informed consent form and there was no conflict of interest among the authors.

Study population

The analyses presented here form part of a larger cross-sectional study on the sociodemographic, lifestyle and health profiles of healthcare students at a higher education institution located in the city of Passo Fundo (RS), Brazil. Every stage of the study was conducted by the students of the scientific methodology course that was offered by the medical school of this institution in the first semester of 2017.

The target population included medicine and dentistry students of a higher education institution. Out of 327 students enrolled in the first semester of 2017 (169 medical students and 158 dental students), 287 participated in this study (148 medical students and 139 dental students), thus resulting in assessment of 87.8% of the total population.

Data collection

The data were collected through a self-administered questionnaire containing closed questions, which was applied during a class to those of the students present who had agreed to participate in the study. This questionnaire was produced based on questions retrieved from a previous study on a similar population: "Drug Use - adaptation of a questionnaire proposed by the World Health Organization (WHO)".[12]

Outcome variable

"Use of psychoactive drugs" was considered to be the outcome. This was evaluated through the question "Do you use psychoactive drugs? (yes or no)".

Regarding descriptive variables, psychoactive drug users were questioned about medical prescriptions (yes, no); purpose of use (relaxation or stress relief, sleep stimulation, sleep suppression, pain control, increased cognitive ability, increased attention for studying, anxiety or depression); duration of drug use (less than six months, six months to one year, one to two years or three years or more); frequency of drug use (daily, at least once a week, at least once a month or rarely); need to increase the dose (yes or no); and side effects (reduced appetite, euphoria, anxiety, tachycardia, irritability, tremors or headache).

Covariables

The variables considered were sex (female or male), age (17 to 20 years old or over 20 years old); marital status (married/cohabitating or separated/widowed/single); course (medicine or dentistry); semester (first to eighth); religious practice (yes or no); living situation (alone or with relatives and/or friends); and expected academic performance (better than or equal to expected or below expected).

Data analysis

In the univariate and bivariate analyses, summary measurements (absolute and relative frequencies) were calculated. To analyze the statistical differences between the categorical variables, Pearson's chi-square test was used, in which the results with P-values lower than 5% (P-value < 0.05) were considered statistically significant. In the multivariate analysis, crude and adjusted odds ratios (OR) and their respective 95% confidence intervals (CI) were estimated according to the exposure variables. Variables with P-value < 0.20 in the bivariate analyses were included in the multivariate models. The data were analyzed using the Statistical Package for the Social Sciences (SPSS) software, version 20.0.

RESULTS

The study population consisted predominantly of female university students aged 20 to 36 years. The population was divided into virtually equal proportions for the dental and medical schools. **Table 1** presents the characteristics of the students who were and were not using psychoactive drugs.

Among the students who were using psychoactive drugs, high frequencies of psychoactive drugs had been prescribed by physicians (95.8%) and with the purpose of relaxation or stress relief (73.2%). Most of these students had been using the drugs for one or two years (35.2%) and were taking them daily (71.9%). It was noted that almost half of the students had needed to increase the dose from the initial drug prescription (49.3%). Many students reported having some side effect (59.2%), such as headaches (23.8%) (**Table 1**).

Table 2 presents the prevalence of psychoactive drug use stratified according to the covariables. Higher prevalence were observed among women, medical students and participants with lower-than-expected academic performance (P-value < 0.05). As anticipated, the same variables were associated with the use of psychoactive drugs in the crude models. Thus, women, medical students (compared with dental students) and participants with lower academic performance were more likely to use psychoactive drugs. After the multivariate adjustments, the variables of "sex", "course" and "school performance" remained significant.

DISCUSSION

The aim of the present study was to estimate the use of psychoactive drugs among healthcare students at a higher education institution located in the city of Passo Fundo (RS), Brazil, and to ascertain the associated demographic and lifestyle factors. It was observed that most of the university students interviewed who were using psychoactive drugs were obtaining them through medical prescription (95.8%). Among these, the majority reported using this type of medication for relaxation or stress relief (73.2%).

For young people today, university is the time for preparation to face the professional world. This is an important step in an increasingly competitive and challenging society. During the academic course, students feel that they are under pressure to succeed and feel the need to continually surpass themselves. Hence, improvement of concentration, memory and attention is the need that leads university students to misuse psychoactive drugs.[8]

Previous studies targeting student populations have indicated that the prevalence rates for the use of stimulants are between 1% and 38%.[8] The reasons for their use that university students have stated include optimization of studies, increased concentration, compensation for sleep deprivation and enhancement of moments of relaxation.[8,13]

According to Wilens et al.,[10] higher education requires a certain level of development of cognitive function and, in medical schools, this level is particularly high. Thus, students use brain stimulants to increase academic performance.

This study showed an association between the use of psychoactive drugs and women. This result agrees with the findings from other studies such as the one by Lemos et al.,[14] which showed that there was higher alcohol consumption among men and higher use of tranquilizers among women. Boskovitz et al.[15] concluded that while men were more likely to consume alcohol, solvents, marijuana and cocaine, women were more likely to use tranquillizers.

According to Simoni-Wastila et al.,[16] there is a pattern of medicalization among adult women, because they seek relief for their domestic or professional problems through "chemical tranquilizers".

The present study also found that psychoactive drug use had an influence on school performance. In a previous study, it was observed that a slight increase in concentration and motivation

Table 1. Characteristics of the population of healthcare students at a higher education institution who were using psychoactive drugs (n = 71) and were not using psychoactive drugs (n = 216), in the city of Passo Fundo (RS), Brazil, 2017

	Using		Not using	
	n	%	n	%
Sex				
Male	13	18.3	63	29.2
Female	58	81.7	153	70.8
Age category (years)				
17 to 20	25	35.2	91	42.1
21 to 36	46	64.8	125	57.9
Course				
Medicine	49	69.0	99	45.8
Dentistry	22	31.0	117	54.2
Semester				
I to III	38	53.5	95	44.0
IV to VIII	33	46.5	121	56.0
Religion				
Non-practicing	14	19.7	29	13.4
Some religious practice	57	80.3	187	86.6
Living situation				
Alone	35	4.93	101	46.8
With relatives/friends	36	50.7	115	53.2
Marital status				
Married/cohabitating	-	-	13	6.0
Separated/widowed/single	48	100	203	94.0
Expected academic performance				
Higher than or equal to expected	30	16.7	7	13.5
Below expected	40	42.3	133	61.6
Drugs prescribed by physicians	41	57.7	57.7	38.4
Yes	68	95.7	-	-
No	3	4.3	-	-
Purpose of use			-	-
Relaxation or stress relief	52	73.2		
Sleep stimulation	8	11.3	-	-
Sleep suppression	1	1.4	-	-
Pain control	5	7.0	-	-
Increased cognitive ability	3	4.3	-	-
Increased attention for studying	2	2.8	-	-
Anxiety	-	-	-	-
Depression	-	-	-	-
Duration of drug use				
Less than 6 months	18	25.4	-	-
6 months to 1 year	6	8.3	-	-
1 to 3 years	25	35.3	-	-
3 years or more	22	31	-	-
Frequency of drug use				
Daily	51	71.9	-	-
At least once a week	10	14	-	-
At least once a month	7	9.8	-	-
Rarely	3	4.3	-	-
Need to increase the dose				
Yes	35	49.3	-	-
No	36	50.7	-	-
Side effects				
Yes	42	59.2	-	-
No	29	40.8	-	-

was achieved through use of psychoactive drugs.[17] A study by Hildt et al.[8] emphasized that there was a scarcity of real observed cognitive effects, given that the students reported that there was an increase in concentration and memory capacity after using such drugs. However, despite this positive subjective experience, few students reported that drug use had any real objective effect on academic outcomes. This raises the hypothesis that there might be a motivational effect associated with psychoactive drugs that is more important than the real effect on individual capacities.[8] In some studies, many students on medications reported having high stress levels, especially before final exams or the due dates for handing in papers.[9,18,19]

In the present study, medical students showed higher prevalence of psychoactive drug use than dental students. In a study conducted by Webb et al.[13] among medical students in the United States, it was suggested that 15% of the students were using stimulants during the academic course. It was found that 83% of the students were using stimulants specifically to improve academic performance.

Several authors have pointed out that the peculiar characteristics of medical schools may be contributing to the increased use of psychoactive substances among students. These characteristics include high course load, responsibility for patient healing, ethical issues, deaths of patients under student care and facilitated access to certain drugs that are restricted to healthcare professionals.[20]

In the present study, most of the students reported using this type of medication for the purpose of relaxation or stress relief (73.2%). Medical school has been described as a source of stress for students, who report especially the loss of personal freedom, excessive academic pressures and feelings of dehumanization.[21] They also complain about the lack of leisure time and the strong competition among colleagues. These and other factors such as contact with sick patients predispose towards appearance of depressive conditions, anxious reactions, obsessive-compulsive neurosis and hypochondria, which lead students to use psychoactive drugs.[22]

Some studies have already proven the high prevalence of stress, anxiety and depression among medical students.[23] According to the study by Mesquita et al.,[24] the students themselves believe that the stress of medical school is a major factor for their use of drugs, particularly the stress relating to competitiveness, the intense course load, the abrupt transition from theoretical activities to practice and the medical residency test.

Medical students in the United States have been found to be more susceptible to developing symptoms of stress and dissatisfaction, and greater severity of signs of psychiatric illnesses.[25,26] Medical students have been selected as the target population in a number of studies, which shows that there is higher concern regarding this specific group, given these individuals' greater knowledge of pharmacology and greater access to these substances.[27-29]

In another study, in which medical students stood out because of their facilitated access to these substances, the euphoric effect associated with recreational use was emphasized as an explanation for non-medical consumption of psychoactive substances.[30]

Consumption of psychoactive drugs has produced social and health problems worldwide, especially because of the increasing

Table 2. Prevalence (%) and crude and adjusted odds ratio (OR) with respective 95% confidence intervals (95% CI) for psychoactive drug use among healthcare students at a higher education institution in the city of Passo Fundo (RS), Brazil, 2017

	Use of psychoactive substances		P-value*	Crude		Adjusted		P-value**
	n	%		OR	95% CI	OR	95% CI	
Sex								
Male	13	18.3	**0.048**	1.00	--	1.00	--	**0.049**
Female	50	81.7		1.83	(0.94-3.58)	2.03	(1.00-4.12)	
Age category (years)								
17 to 20	25	35.2	0.18	1.00	--	--	--	0.441
21 to 36	46	64.8		0.74	(0.42-0.30)	--	--	
Course								
Dentistry	22	31.0	**0.001**	1.00	--	1.00	--	**0.010**
Medicine	49	69.0		2.63	(1.48-4.65)	2.35	(1.23-4.49)	
Semester								
I to III	38	53.5	0.104	1.00	--	--	--	0.442
IV to VIII	33	43.5		1.46	(0.85-2.51)	--	--	
Religion								
Non-practicing	14	19.7	0.137	1.00	--	--	--	
Some religious practice	57	80.3		1.58	(0.78-3.20)	--	--	
Expected performance								
Higher than or equal to expected	30	42.3	**0.003**	1.00	--	1.00	--	**0.039**
Below expected	41	57.7		0.64	(0.26-0.78)	1.872	(1.03-3.23)	

prevalence of these drugs.[14] Therefore, there is a need to implement interventions and increase student awareness about the potential effects produced by these substances. It is also crucial for this issue to be addressed in healthcare courses, because students can be considered to be an at-risk group regarding the use of psychostimulants and, in the future, they may be faced with this situation as professionals.[31]

According to Dal Pizzol et al.,[32] the guidelines for public actions to prevent drug misuse should include educational campaigns targeted to the youth population with an emphasis on the most common psychoactive drugs. In addition, control over sales to minors and sales that are contrary to other restrictions established through health surveillance legislation should be reinforced.

One of the main areas of intervention will lie within improving the information and awareness of this student population regarding psychoactive drugs and their clinical and ethical effects, so as to prevent use and decrease the number of users.

Few scientific data on the effects of psychoactive substances or even on their long-term use have been published in the literature. Many of these studies have only indicated the growing trend in use among young people, without estimating further side effects, addictions or pathological conditions. Precisely because of the lack of data, the aim in this study was to ascertain the degree of use of psychoactive drugs among young students, in order to correlate this with deficiencies in their academic performance. Additionally, the aim was to observe whether the students were under medical care or were self-medicating, which is another matter of controversy within the academic environment. From analysis on the results, a high prevalence of psychoactive drug use was found, thus corroborating the scientific evidence.

The present study may have provided an important tool for further scientific studies focusing on the academic population of university courses with high course loads. From reflection on the increasing use of psychoactive drugs, it can be noted that this topic requires greater attention from the scientific community, to augment the sparse literature that currently exists and to obtain further data and reach more conclusions on this topic.

Therefore, it is necessary to implement interventions and increase the population's awareness of the potential effects produced by psychoactive substances. Considering that the participants in the present study were healthcare students, it was expected that the consumption of stimulants would be lower, because such students are aware of the risks of these substances. However, what was most striking to us was the fact that most of these users had a medical prescription for the use of such drugs.

CONCLUSION

From the results obtained, it was possible to conclude that:

- The use of psychoactive drugs among healthcare students at the higher education institution investigated was high, even though this use was via medical prescription.
- There was an association between the use of psychoactive drugs and some factors, with higher prevalence among women, medical students and subjects with lower-than-expected academic performance.

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Relationships between Bloom's taxonomy, judges' estimation of item difficulty and psychometric properties of items from a progress test: a prospective observational study

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ABSTRACT

BACKGROUND: Progress tests are longitudinal assessments of students' knowledge based on successive tests. Calibration of the test difficulty is challenging, especially because of the tendency of item-writers to overestimate students' performance. The relationships between the levels of Bloom's taxonomy, the ability of test judges to predict the difficulty of test items and the real psychometric properties of test items have been insufficiently studied.

OBJECTIVE: To investigate the psychometric properties of items according to their classification in Bloom's taxonomy and judges' estimates, through an adaptation of the Angoff method.

DESIGN AND SETTING: Prospective observational study using secondary data from students' performance in a progress test applied to ten medical schools, mainly in the state of São Paulo, Brazil.

METHODS: We compared the expected and real difficulty of items used in a progress test. The items were classified according to Bloom's taxonomy. Psychometric properties were assessed based on their taxonomy and fields of knowledge.

RESULTS: There was a 54% match between the panel of experts' expectations and the real difficulty of items. Items that were expected to be easy had mean difficulty that was significantly lower than that of items that were expected to be medium ($P < 0.05$) or difficult ($P < 0.01$). Items with high-level taxonomy had higher discrimination indices than low-level items ($P = 0.026$). We did not find any significant differences between the fields in terms of difficulty and discrimination.

CONCLUSIONS: Our study demonstrated that items with high-level taxonomy performed better in discrimination indices and that a panel of experts may develop coherent reasoning regarding the difficulty of items.

INTRODUCTION

Assembling a knowledge test can be a challenging task, especially with regard to calibrating the difficulty of the test. Although many studies have addressed how useful experts' opinions can be, their predictions of the difficulty is often different from what the students perceive. This uncertainty relates to the multiple factors involved in the cognitive process that is necessary for answering a question and to the tendency of item-writers to overestimate students' performance.[1,2] Questions can require lower or higher levels of cognitive processing, depending on whether students have to recall, minimally understand or apply their knowledge. Although studies have investigated experts' predictions and the requirements for cognitively processing the items, little attention has been paid to the combination of these two factors. Knowing whether there are relationships between the type of cognitive processing that the item requires, experts' predictions and the difficulty of the items may help experts to predict the difficulty of knowledge tests better.

Bloom's taxonomy of educational objectives was designed to classify the learning objectives, skills and abilities that are expected from learners at the end of an educational program.[3,4] Educational objectives may range from memorization of knowledge to creation of new knowledge in an increasingly complex and hierarchical fashion.[3,5] Within this framework, cognitive processing is represented as a cumulative hierarchy that is made up of lower and higher levels of acquired

knowledge. There are two low levels, which relate to remembering and minimally understanding the knowledge. There are two intermediate levels (third and fourth levels), which relate to applying the knowledge to a new situation and making connections between ideas (analyses). There are two high levels, which relate to justifying decisions (evaluations) and creation of new knowledge. In theory, mastery of lower levels is required in order to attain higher levels.

Questions that assess higher levels of complexity of knowledge are difficult to produce, and there is a debate regarding whether multiple-choice questions have the capacity to assess higher levels of complexity, i.e. situations of creation of new knowledge.[6] More importantly, higher-order cognitive processing has been shown to improve students' knowledge retention, compared with low-order cognitive processing. Additionally, medical practice requires the use of higher-order cognitive processing more than lower-order processing. Although there is a trend within medicine towards assessing students at higher levels of cognitive processing, little attention has been paid to Bloom's taxonomy when setting pass/fail scores.

Setting pass/fail scores is the main concern in educational assessment.[7,8] There are two main categories of procedures for setting standards: norm-referenced (relative) and criterion-referenced (absolute). Relative methods take the results from the test into account to set the standards. They help rank the examinees but may lead to a large variation in the cutoff scores and are poorly accepted in some cultures. Absolute methods are widely used worldwide, but they face several criticisms because they lead to large variation in failure rates and do not consider the different difficulties between different exams.[9,10]

One example of a criterion-referenced method that is often used within medical education is the Angoff method. In this method, the judges of the examination estimate the percentage of borderline examinees who will respond correctly to the test items. The judges' estimates are then averaged for each item, and the cutoff is set as the sum of the averages.[11]

Progress tests have been used in Brazilian schools for more than fifteen years.[12-14] They have been gaining greater attention over the last five years because of the Brazilian Association of Medical Education's efforts to improve the quality of medical students' evaluations throughout the country.[15] Therefore, progress tests give rise to a good opportunity for studying the psychometric properties of assessment items.

OBJECTIVE

Although some studies have analyzed the application of Bloom's taxonomy to test items[16] and the utility of Angoff methods using standard settings,[17,18] the relationship between these two has not been extensively examined. In the current study, we investigated the relationships between the exam judges' estimates (through an adaptation of the Angoff method) and the classification of the difficulty and discrimination levels of items, using Bloom's taxonomy in a progress test setting.

METHODS

Study design

For this prospective observational study, data from the 2018 progress test from a consortium of ten Brazilian medical schools, mainly in the state of São Paulo, Brazil, were examined. Our examination of the progress test was designed to assess the knowledge that final-year medical students should have, in order to provide feedback to medical students and institutions.[15] All the students at these ten schools underwent the same test once a year, on the same day, at the same time. The students had four hours to complete the test, and after two hours had elapsed, they could use the question booklet of the test for self-study purposes. Written feedback with commentary and bibliographic references for each item was provided a few days after the test.

A blueprint for the progress test was developed by the consortium, consisting of six fields of knowledge: basic science, internal medicine, pediatrics, surgery, obstetrics and gynecology, and public health. Every year, the coordinators of the progress test create a set of orders for items that address the blueprint. Each school is represented at the meetings by an academic staff member. This representative is responsible for the exchange of information between his school and the others, as well as for delivering the orders to his colleagues, who will be responsible for writing the required items. A single order from the coordinators might therefore consist of up to ten written items. Afterwards, several specialists from the consortium schools hold a meeting to select the items that will make up the final exam: 20 items for each field, thus totaling 120 multiple-choice items, each presenting four alternative responses. Any unused items are stored in a database.

Bloom's taxonomy classification of the items

The items were classified in accordance with the levels of cognitive domains that were proposed by Bloom, as revised by Anderson and Krathwohl.[5] Here, items focusing on remembering and developing minimal understanding of knowledge were classified as the lowest taxonomy level; items focusing on knowledge application and analysis were classified as the intermediate taxonomy level; and items focusing on synthesis and evaluation were classified as the highest taxonomy level. These items were classified by two experts, who classified the items in accordance with their use in tests over the past five years.

Angoff adaptation

In this study, the panel of experts was asked to set the expected difficulty for each item selected. The difficulty would be estimated by considering the performance of a sixth-year medical student. In the original use of the Angoff method, the expected percentage of correct answers among the examined population was ascertained.[19] Here, we asked the experts to classify the items as follows: difficult (expectation that more than 80% of the answers would be incorrect), medium (expectation that 40% to 80% would be incorrect), and easy (expectation that less than 40% would be incorrect). The expected level of difficulty of the items was developed based on an agreement that was reached after a discussion among the judges.

Statistical analysis

A specialized institution marked the tests and performed psychometric analysis on the items by focusing on their difficulty, the discrimination index and biserial correlation. This last aspect will not be discussed further in the present study. For the purpose of the present study, test responses that consisted of guessing constant answers were excluded from the analysis (i.e. proportion of correct answers < 25%). We only used the data from the sixth-year students at the ten medical schools.

As described above, items with a difficulty index greater than 0.8 were considered difficult, items with indices lower than 0.4 were considered easy and items with indices between 0.4 and 0.8 were considered medium.

The normality of the data was tested using the Shapiro-Wilk test. The differences in mean values were tested using single-factor analysis of variance (ANOVA) followed by the Tukey post-test for the parametric data; or using the Kruskal-Wallis test followed by the Dunn test for the nonparametric data. Correlations between the different data were made using the Spearman correlation test. We set the statistical significance level at a P-value of 0.05.[20] The statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS), version 24.0, and the BioEstat software, version 5.0.

Ethical considerations

Since we dealt with secondary data and no student was identified, ethics committee approval was not necessary.

RESULTS

A total of 4,596 students participated in the test (94.1% of the total population), from which 4,563 were included in the general psychometric analysis. Of these, 771 students were in their sixth year (**Table 1**). One item relating to obstetrics and gynecology was invalidated due to inconsistent answers, and therefore, 119 items were analyzed.

Bloom's taxonomy

The 119 items were classified using Bloom's taxonomy. Of these, 52 (43.7%) had high-level taxonomy, 32 (26.9%) had medium-level taxonomy and 35 (29.4%) had low-level taxonomy. More than 50% of the items relating to internal medicine, pediatrics, surgery and obstetrics and gynecology were classified as presenting high-level taxonomy, whereas most of the items relating to basic sciences and public health were classified as presenting low-level taxonomy. The distribution of the items was significantly different between the fields ($P < 0.001$), such that public health presented higher frequency of items with low-level taxonomy, compared with internal medicine, pediatrics, surgery and obstetrics and gynecology. In addition, the distribution of items was statistically different between pediatrics and basic sciences (**Figure 1**). **Table 2** presents the distribution of items according to their taxonomy among the fields of knowledge.

Item difficulty

The panel of experts judged 62 items as easy, 41 as medium and 16 as difficult. Based on the analysis of the real difficulty of the items, 79 items were easy, 82 were medium and only one item was difficult (**Figure 2**). For 65 items (54%), the expected difficulty was the same as the difficulty in reality; 13 items (11%) were underestimated (i.e. they were more difficult than expected); and 41 items (34%) were overestimated (i.e. they were easier than expected). The rates of concordance between expected difficulty and difficulty in reality were 60% for basic sciences, pediatrics and public health; 50% for internal medicine and surgery; and 47% for obstetrics and gynecology.

Table 1. Summary of the students who sat the examination, according to school and undergraduate year

School	1st year	2nd year	3rd year	4th year	5th year	6th year
UNICAMP	117	112	113	115	122	125
UNESP	90	91	85	90	83	88
USP-RP	93	96	93	92	95	111
USP-BA	59	0	0	0	0	0
UNIFESP	116	124	115	111	117	127
UFSCAR	39	41	40	33	37	43
FAMEMA	79	78	71	80	75	79
FAMERP	74	81	74	76	79	62
UEL	79	79	73	78	61	72
FURB	72	82	76	70	69	64
Total	**818**	**784**	**740**	**745**	**738**	**771**

UNICAMP = Universidade Estadual de Campinas; UNESP = Universidade Estadual Paulista; USP-RP = Universidade de São Paulo-Ribeirão Preto; USP-BA = Universidade de São Paulo-Bauru; UNIFESP = Universidade Federal de São Paulo; UFSCAR = Universidade Federal de São Carlos; FAMEMA = Faculdade de Medicina de Marília; FAMERP = Faculdade de Medicina de São José do Rio Preto; UEL = Universidade Estadual de Londrina; FURB = Fundação Universidade Regional de Blumenau.

The analysis on the difficulty of the items in reality according to the levels of difficulty set by the panel experts demonstrated mean difficulties of 0.28, 0.37 and 0.49, for items considered easy, medium and difficult, respectively. These differences were statistically significant (F = 8.604; P < 0.01): the items that were considered easy presented mean difficulty significantly lower than that of the items considered medium (P < 0.05) and the items considered difficult (P < 0.01).

Obstetrics and gynecology and basic sciences were the categories with the highest mean difficulty, followed by internal medicine, surgery, pediatrics and public health (**Table 2**). We did not find any significant differences between the fields of knowledge (F = 0.323; P = 0.898), although there was a trend towards public health to be considered easier.

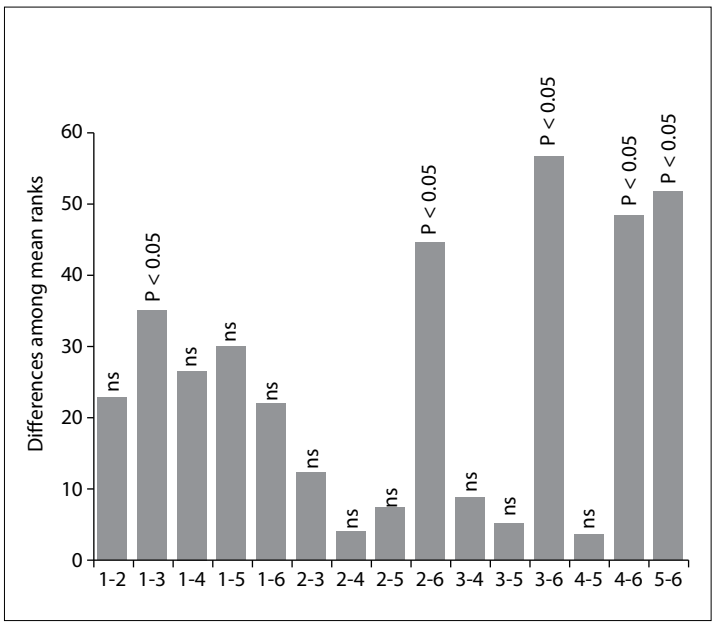

Figure 1. Differences among the mean ranks of the fields of knowledge, in accordance with the classification of Bloom's taxonomy. 1: basic sciences; 2: internal medicine; 3: pediatrics; 4: surgery; 5: obstetrics and gynecology; 6: public health. ns: non-significant. Public health was significantly different from internal medicine, pediatrics, surgery and obstetrics and gynecology. Pediatrics was also significantly different from basic sciences. Overall, P < 0.0001.

The mean difficulties of the items classified as having low, intermediate and high-level taxonomies were 0.29, 0.34 and 0.36, respectively. We did not find any significant differences between the levels of taxonomy regarding difficulty (F = 0.993; P = 0.374), and we did not find any correlation between the taxonomy of the items and their difficulty (rho = 0.172; P = 0.06).

Item discrimination

The mean discrimination indices were 0.38 for obstetrics and gynecology; 0.32 for pediatrics, surgery and internal medicine; 0.31 for public health; and 0.27 for basic sciences (**Table 2**). Although obstetrics and gynecology demonstrated a trend towards greater discrimination, we did not find any significant differences between the fields of knowledge (H = 8.734; P = 0.12).

Comparison of discrimination between the items according to their taxonomy group demonstrated mean discrimination indices of 0.28, 0.31 and 0.35 for items with low, intermediate and high levels of taxonomy, respectively. A statistical difference was found between the groups with low and high levels of taxonomy (P = 0.026; **Figure 3**). A Spearman correlation test demonstrated that there was a positive correlation between the taxonomy of the items and their discrimination indices (rho = 0.25; P = 0.006).

DISCUSSION

This study sought to use progress tests to investigate the relationships between the difficulties and discrimination and the judges' estimates of exam items, through an adaptation of the Angoff method; and to classify them using Bloom's taxonomy. Items with higher-level taxonomy had higher discrimination indices than those with lower-level taxonomy. We also found that items that were expected to be easy were indeed easier than items that were expected to be difficult.

At the end of medical school, students are expected to demonstrate high-order cognitive processes. For example, students in the initial years of training perform better in questions with lower-level taxonomy, whereas students in their final years perform better in relation to items with higher-level taxonomy.[21] In our test, items with higher-level taxonomy predominated, which was expected because the test was designed to include vignette-based items.

Table 2. Summary of psychometric properties and distribution of Bloom's taxonomy according to the fields of knowledge of the exam

Area	Mean difficulty	Mean discrimination	High-level taxonomy	Medium-level taxonomy	Low-level taxonomy
Basic sciences	0.36	0.27	20%	35%	45%
Internal medicine	0.35	0.32	50%	35%	15%
Pediatrics	0.32	0.32	75%	15%	10%
Surgery	0.34	0.32	55%	35%	10%
Obstetrics and gynecology	0.36	0.38	63%	26%	11%
Public health	0.29	0.31	0%	15%	85%
Total	**0.34**	**0.32**	**44%**	**27%**	**29%**

In addition, tests with higher-level taxonomy had better discrimination indices than tests with lower-level taxonomy. These data emphasize the need to develop tests for better discrimination of items with high-level taxonomy. In this regard, case-based questions might be more suitable for higher-order cognitive processing[12] and consequently might be more appropriate for tests that are designed to assess the knowledge of final-year students.

Interestingly, in the field of public health, the indices of discrimination and difficulty tended to be lower. This can possibly be explained in terms of the predominance of lower-order cognitive processes that are involved in the items from this subject. These findings may relate to the characteristics of this field: students are required to have sufficient knowledge of legislation and conceptual frameworks.

Although the test was easier than estimated by the judges, the mean values for the total score were in accordance with those found in other studies on progress testing data.[22-24] While the low achievement of students at the final-year level creates doubt regarding the unrealistic expectations of item writers and the quality of the items,[25] discussion of the underestimated items can be useful for medical schools and their academic staff as a means for monitoring the educational environment.

The panel of experts demonstrated coherent reasoning in classifying the difficulty of the items. In addition, the group analysis indicated that the items that were expected to be easy presented lower mean difficulty indices than the items that were expected to be medium or difficult; while items that were expected to be medium had lower mean difficulty indices than items that were expected to be difficult (although these differences were not statistically significant).

Similarly, Kibble and Johnson found coherence between the intended and actual difficulty of the items, with a successful estimation rate of 48%.[26] Conversely, they did not find any correlation between the taxonomy of the test items and their difficulty and discrimination indices. These authors placed doubt on the usefulness of efforts for estimating the difficulty of items and their taxonomy as a means for controlling examination difficulty. This may have been due to the tendency of the item writers to overestimate the students' performance, and to the fact that item writers and examinees approach the same material in different ways, based on different levels of knowledge.[2,27] Corroborating this hypothesis, Verhoeven et al. found that using recent graduates as judges for setting progress testing standards had good reliability and credibility, and subsequently found that the data from recent graduates were more credible than data from item writers, regarding their estimates as judges.[17,18]

The present study had some limitations: firstly, we used only one edition of the progress test, and the number of items analyzed was limited. Continuous monitoring of the items applied by our consortium may strengthen our findings. Secondly, this was the first time that we had used the Angoff method to examine the progress test, which means that the calibration of the judges may not have been accurate. Thirdly, in our adaptation of the Angoff method, we did not perform an individual analysis on each judge's estimations. Future development of this research should involve repetition of the proposed Angoff modification, to test its validity and reliability across different tests. Nonetheless, despite these limitations, our study demonstrated novel highlights regarding the better performance of items with high-level taxonomy, for obtaining better discrimination indices, and the high degree of precision of the panel of specialists regarding estimation of the difficulty of exam items.

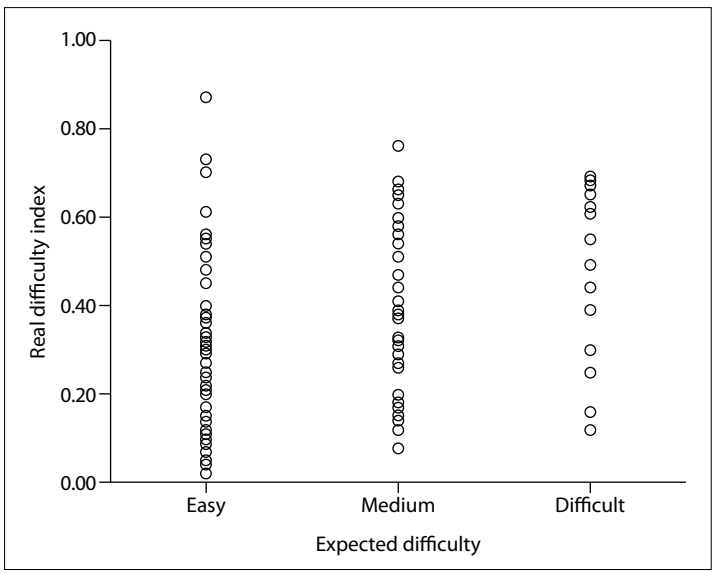

Figure 2. Scatter diagram illustrating the indices of real difficulty of the items, according to their classification by the panel of judges.

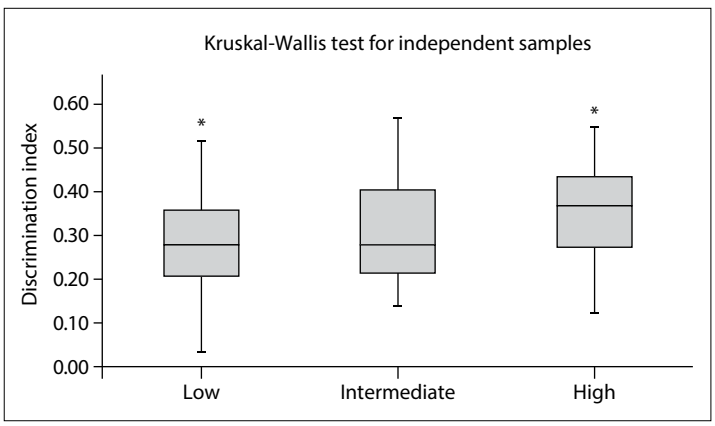

Figure 3. Differences between the taxonomy groups regarding mean discrimination indices. The items with low-level taxonomy had significantly lower discrimination indices than the items with high-level taxonomy (P = 0.026).

Currently, item response theory is used to compose exams using previously tested items.[28,29] Despite the advantages of this method, it has limited usefulness with regard to new written items. Our data suggest that classification of items using Bloom's taxonomy (which can be performed prior to application of the exam) can select the items with better discrimination performance. Lastly, future research could provide correction formulas based on the judges' expectations, in order to better predict the real difficulty of the items.

CONCLUSION

In conclusion, the items with higher-level taxonomy provided better discrimination of the students' performance; and the panel of experts demonstrated that they coherently deduced the difficulty of the exam items.

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Synergic effect of simvastatin in combination with amphotericin B against environmental strains of *Cryptococcus neoformans* from northeastern Brazil: a prospective experimental study

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KEY WORDS (MeSH terms):
Cryptococcosis.
Cryptococcus neoformans.
Drug resistance, fungal.
Antifungal agents.
Drug interactions.

AUTHORS' KEY WORDS:
Cryptococcosis treatment.
HIV infection.
Fractional inhibitory concentration index.
Antifungal therapy.

BACKGROUND: Statins are used as cholesterol-lowering drugs and may also have direct antimicrobial effects.

OBJECTIVE: To evaluate synergic interactions between simvastatin and both amphotericin B and fluconazole, against environmental strains of *Cryptococcus neoformans* isolated from captive birds' droppings.

DESIGN AND SETTING: Experimental study conducted at Federal University of Piauí, Parnaíba, in collaboration with Federal University of Triângulo Mineiro, Uberaba, Brazil.

METHODS: Statin susceptibility tests of *Cryptococcus neoformans* samples were performed as prescribed in standards. Interactions of simvastatin with amphotericin and fluconazole were evaluated using the checkerboard microdilution method. Presence of these interactions was quantitatively detected through determining the fractional inhibitory concentration index (FICI).

RESULTS: Isolates of *Cryptococcus neoformans* were obtained from 30 of the 206 samples of dry bird excreta (14.5%) that were collected from pet shops and houses. Ten isolates were selected for susceptibility tests. All of them were susceptible to amphotericin and fluconazole. All presented minimum inhibitory concentration (MIC) > 128 µg/ml and, thus, were resistant *in vitro* to simvastatin. An *in vitro* synergic effect was shown through combined testing of amphotericin B and simvastatin, such that six isolates (60%) presented FICI < 0.500. Two isolates showed considerable reductions in MIC, from 1 µg/ml to 0.250 µg/ml. No synergic effect was observed through combining fluconazole and simvastatin.

CONCLUSION: These results demonstrate that simvastatin should be considered to be a therapeutic alternative, capable of potentiating the action of amphotericin B. However, further studies are necessary to clarify the real effect of simvastatin as an antifungal agent.

INTRODUCTION

Cryptococcus neoformans is an encapsulated and opportunistic yeast fungus that has worldwide distribution. In the urban environment, it is often found in soil contaminated with dried bird excreta, and thus can infect humans and other animals through inhalation.[1,2]

Currently, the treatment for cryptococcosis is based on use of amphotericin B alone or in combination with 5-flucytosine or azoles, in the early stages of the disease. However, the high toxicity associated with these antifungals may restrict their use in special clinical settings, such as in cases of chronic kidney diseases. In situations of secondary prophylaxis, use of fluconazole is indicated to minimize the risk of recurrence of infection in patients who are still in an immunosuppressive state.[3] Treatment of cryptococcosis using azoles (e.g. fluconazole) requires long periods and can favor the emergence of microbial resistance. Although azoles are less toxic than polyenes, they present low efficacy for the initial treatment of the disease. In contrast, amphotericin B provides a more effective therapeutic response against the fungus, although its use should be limited because of its toxicity.[2]

Emergence of antifungal resistance among environmental and clinical isolates of *Cryptococcus neoformans* has been described over the last few decades. Prolonged use of antifungals for treating

patients with immunosuppressive disorders such as acquired immune deficiency syndrome (AIDS) may contribute towards antifungal resistance.[4-6]

Development of new therapeutic strategies is therefore extremely important, given the low number of antifungals available and the scarcity of these drugs in limited-resource settings. In this context, new drugs can contribute towards treatment of cryptococcosis through enhancing the effect of traditional antifungals.[7]

Statins are drugs that are used to treat cardiovascular diseases relating to high cholesterol levels in humans. The mechanism of action of statins is based on inhibition of 3-hydroxy-3-methyl-glutaryl-CoA reductase (HMG-CoA), an enzyme responsible for liver cholesterol biosynthesis. As this enzyme becomes inhibited by statins, the pathway of cholesterol synthesis is blocked, thus resulting in a decrease in LDL cholesterol levels. Additionally, this enzyme synthesizes mevalonic acid, an important precursor in the synthesis of sterols such as ergosterol in fungi and cholesterol in humans.[8]

Hence, statins are important inhibitors of precursors of the synthesis of these sterols. Several studies have demonstrated that some statins present antifungal activity: for example, fluvastatin and simvastatin against species of *Candida* and *Cryptococcus*. Therefore, combining statins with antifungal agents could decrease the length of time for which infected patients are exposed to toxic drugs. Consequently, this would reduce the side effects from use of antifungal agents, especially in cases of emergence of fungal strains that are resistant to conventional treatment.[9,10]

In this context, given the increasing numbers of cases of antifungal resistance and the toxicity presented by the conventional treatment scheme, the antifungal potential of simvastatin alone and in association with amphotericin B and fluconazole was tested on *Cryptococcus* isolates that were recovered from the dried feces of captive birds in northeastern Brazil.

METHODS

Ethics statement

This study was approved by the Research Ethics Committee of the Federal University of Piauí (Universidade Federal do Piauí, UFPI), Campus Ministro Reis Velloso, Parnaíba, Piauí, Brazil, under the number CAAE 45296315.5.0000.5669, on July 10, 2015.

Environmental samples

Between September 2015 and April 2017, a total of 206 dry fecal samples from captive birds of the species *Columbia livia, Melopsittacus undulatus* and *Lonchura striata* were collected from pet shops and houses located in different districts of Parnaíba, Piauí, Brazil. This number of samples comprised the total number available for collection at the time of the study. The dried feces were picked up from the birdcages using sterile swabs and were inoculated into tubes containing 10 ml of sterile saline solution with 0.4 g/l of chloramphenicol. It was not possible to determine how long these samples had been exposed before the cleaning procedures were performed.

Isolates of *Cryptococcus neoformans* were obtained from 30 of the 206 samples of dry bird excreta (14.5%) that had been collected from pet shops and houses. Ten of these isolates were selected for susceptibility tests because all the 30 isolates belonged to the same molecular type.

Each of the 206 feces samples was placed in a separate tube (thus, there were 206 tubes). The tubes were shaken to mix the contents for two minutes and the samples were then left to settle at room temperature for 30 minutes. Next, 100 ml of the supernatant was inoculated onto Niger seed (*Guizotia abyssinica*) agar plates supplemented with 0.4 g/ml of chloramphenicol. Each sample was spread on four plates, incubated at 35 °C and examined daily for 10 days, macroscopically, in order to identify any presence of smooth, beige to dark brown colonies suggestive of *Cryptococcus* spp.[11]

These colonies were streaked onto Sabouraud agar at 35 °C for 48 hours and were identified using the India ink test and urease. Canavanine-glycine-bromothymol blue (CGB) medium was used to differentiate *Cryptococcus neoformans* from *Cryptococcus gattii*. The isolates thus obtained (which were clones) were then stored until the tests were performed. To ensure preservation, some of the cell masses were stored in distilled water at 4 °C, while others were stored in yeast peptone dextrose broth (Difco Laboratories, Sparks, MD, USA) with 30% glycerol at 4 °C.

Deoxyribonucleic acid extraction

Deoxyribonucleic acid (DNA) extraction was performed as previously described by Bolano et al.[12] Briefly, a single colony was spread on yeast malt agar (Difco Laboratories) at 37 °C for 72 hours. Then, a loopful of cells from the culture was transferred to Eppendorf tubes and incubated at -20 °C overnight.

Next, 500 ml of cell lysis buffer [1.25 mol/l NaCl, 0.5% sodium dodecyl sulfate (SDS), 100 mmol/l Tris-hydrochloride (Tris-HCl) at pH 7.5 and 0.25 mol/l ethylenediamine tetraacetic acid (EDTA) at pH 8.0] and 5 ml of 2-mercaptoethanol (Sigma, Steinheim, Germany) were added. The tubes were vortexed for 2 minutes and incubated at 65 °C for 1 hour. Then, 500 ml of phenol-chloroform-isoamyl alcohol (25:24:1) was added, shaken for 2 minutes at room temperature and centrifuged at 8150 g, at 4 °C for 15 minutes.

To precipitate the genomic DNA, the aqueous phase was transferred to a new tube and 500 ml of isopropanol alcohol was added and this mixture was incubated at -20 °C overnight. The solution was centrifuged at 4 °C for 15 minutes at 8150 g to pellet the DNA and was then washed with ice-cold 70% ethanol. It was again centrifuged as above and was then air-dried.

The DNA was resuspended in 500 ml of Tris-EDTA (TE) buffer (10 mmol/l Tris-HCl at pH 7.5 and 0.5 mol/l EDTA at pH 8.0) containing 50 mg/ml of ribonuclease-A (RNAse-A) (Invitrogen, Carlsbad, CA, USA). It was then incubated at 37 °C for 30 minutes and stored at 4 °C for polymerase chain reactions (PCR) to be performed.

Restriction fragment length polymorphism (RFLP) of *URA5* gene

Amplification of the *URA5* gene was carried out in a final volume of 25 µl. Each reaction contained 10 µg of DNA, 2.5 µl of 1x PCR buffer (10 mmol/l Tris-HCl at pH 8.3, 50 mmol/l KCl and 1.5 mmol/l $MgCl_2$), 0.2 mmol/l each of deoxyadenosine triphosphate (dATP), deoxycytidine triphosphate (dCTP), deoxyguanosine triphosphate (dGTP) and deoxythymidine triphosphate (dTTP), 1.25 U of Taq DNA polymerase (Invitrogen, São Paulo, Brazil) and 25 ng of each primer: *URA5* (5'-ATGTCCTCCCAAGCCCTCGACTCCG-3') and *SJ01* (5'-TTAAGACCTCTGAACACCGTACTC-3').

From this, a fragment of approximately 800 bp was produced. Thirty-four PCR cycles were performed in a PTC-100 thermocycler (MJ Research Inc., Watertown, MA, USA), consisting of initial denaturation at 94 °C for 4 minutes, followed by denaturation at 94 °C for 45 seconds, annealing at 57 °C for 1 minute, extension at 72 °C for 1 minute and final extension cycle at 72 °C for 10 minutes.

The amplified products were mixed with an equal volume of 2x loading buffer (0.5% bromophenol blue, 0.5% xylene-cyanol and 60% glycerol) and then separated by means of electrophoresis on 1.5% agarose gel (Invitrogen, Barcelona, Spain) in 1x Tris-acetate-EDTA (TAE) buffer at 90 V for 2 hours. They were then stained with 0.5 mg/ml ethidium bromide and viewed under UV light.

Subsequently, 15 µl of PCR products were double-digested using *Sau*96I (10 U/µl) and *Hha*I (20 U/µl) at 37 °C for 3 hours, and then separated by means of electrophoresis on 3% agarose gel at 90 V for 3 hours. RFLP patterns were assigned visually by comparison with the patterns obtained from the standard reference strains (VNI, WM-148; VNII, WM-626; VNIII, WM-628; VNIV, WM-629; VGI, WM-179; VGII, WM-178; VGIII, WM-175; and VGIV, WM-779).[13] In all the reactions, the reference strain of *C. neoformans* ATCC 90112 (American Type Culture Collection, Manassas, VA, USA) was included as a positive control.

Minimum inhibitory concentration (MIC) and fractional inhibitory concentration index (FICI)

The broth microdilution test was performed in accordance with the prescriptions of the Clinical and Laboratory Standards Institute (CLSI). The incubation temperature was changed from 37 °C to 33 °C in order to standardize the growth of the strains.

Fluconazole (Zoltec, Pfizer, Guarulhos, SP, Brazil) was initially dissolved in sterile water in accordance with document M27A3 of the CLSI.[14] Amphotericin B (Sigma, São Paulo, Brazil) was diluted in dimethyl sulfoxide (DMSO) (Vetec, Brazil). The serial dilutions of the antifungal agents were prepared in RPMI 1640 medium (with L-glutamine and without sodium bicarbonate) (MP Biomedicals, France), and were buffered to pH 7.0 with 0.165 mol/l morpholinopropane sulfonic acid (MOPS) (Êxodo Scientific, Brazil). The fluconazole concentration ranged from 0.0625 to 64 µg/ml and the final concentrations of amphotericin B ranged from 0.0312 to 16 µg/ml. The *Candida krusei* strain ATCC 6258 were used as a quality control.

The minimum inhibitory concentration (MIC) results were defined for *C. neoformans* as specified by the CLSI[14] and by other authors, as follows: MIC > 64 µg/ml was deemed to be resistant; MIC between 16 and 32 µg/ml was considered to be susceptible dose-dependent (SDD); and MIC < 8 µg/ml was considered to be susceptible.[15,16]

The interactions of simvastatin (Pharmanostra Ltda., São Paulo, Brazil) with amphotericin and of simvastatin with fluconazole were evaluated by means of the checkerboard microdilution method.[17] The stock solution of simvastatin was prepared with DMSO at a concentration of 12800 µg/ml and was then diluted in RPMI 1640 medium buffered with MOPS in the proportions of 1:50. The final concentrations in the wells ranged from 128 to 0.25 µg/ml. The interactions between these drugs were evaluated quantitatively by determining the fractional inhibitory concentration index (FICI). They were classified as synergistic if FICI ≤ 0.500, indifferent if FICI > 0.500-4.0 and antagonistic if FICI > 4.0.[17,18] All the tests were performed in duplicate and on different days.

RESULTS

Isolates of *Cryptococcus* spp. were recovered from 30 of the 206 samples of dried feces from captive birds that were collected from pet shops (14.5%). According to the bird species, the isolates of *Cryptococcus* spp. were recovered as follows: 28 (93.3%) from *Columbia livia*, one (3.3%) from *Melopsittacus undulates* and one (3.3%) from *Lonchura striata domestica*. All of the isolates presented capsules, urease production, growth at 37 °C and melanin production on Niger seed agar. In addition, all of them presented a negative canavanine glycine bromothymol blue (CGB) test. According to the molecular typing, all the isolates of *Cryptococcus neoformans* were identified as VNI (**Figure 1**).

Ten isolates were selected to be subjected to the susceptibility tests. All of them presented susceptibility to amphotericin and fluconazole. On the other hand, all of these isolates presented MIC > 128 µg/ml in relation to simvastatin and, thus, were considered resistant *in vitro* to this drug.

In the test on amphotericin B and simvastatin in combination, 6 (60%) of the 10 isolates studied presented FICI < 0.500, thus revealing a synergistic *in vitro* effect. Moreover, 3 samples (30%) presented FICI values of 0.501, i.e. only slightly above the cutoff value for synergism. Synergism between simvastatin and amphotericin B was detected at a simvastatin concentration of 0.250 µg/ml. The isolates PI1543 and PI16202 showed MIC reductions from 1 µg/ml to 0.250 µg/ml, thus indicating clearly the synergistic effect of combining simvastatin with amphotericin B. However, when simvastatin was combined with fluconazole, no synergistic effect was observed (**Table 1**).

DISCUSSION

In the present study, *Cryptococcus neoformans* was isolated from 14.5% of the samples collected. Similar results were found in some other regions of Brazil: *Cryptococcus neoformans* was isolated in 17.3% of the captive birds droppings in a study in the city of Uberaba, Minas Gerais, and in 18.5% in the city of Salvador, Bahia.[19,20] However, in other regions of Brazil, the prevalence has differed, ranging 25.3% in the southern region to 50% in the central region of this country.[21] The variation in the isolation rates can be related to climatic factors, since high temperatures inhibit fungal growth.[22] Additionally, the methodology

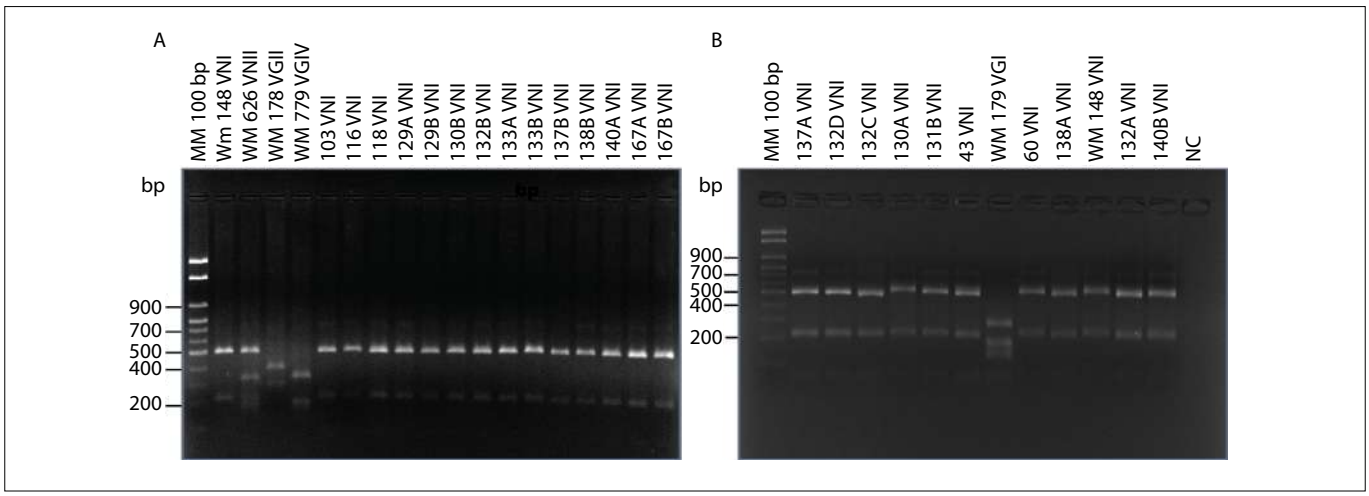

Figure 1. A: Representative agarose gel electrophoresis of *URA5* polymerase chain reaction-restriction fragment length polymorphism showing the identification of molecular types VNI, VNII, VGII and VGIV (control samples) and the environmental *Cryptococcus neoformans* isolates of this study, all of genotype VNI (columns 6 to 19). MM: 100 bp molecular marker (column 1). B: Representative agarose gel electrophoresis of *URA5* polymerase chain reaction-restriction fragment length polymorphism showing the identification of molecular types VNI and VGI (control samples) and the environmental *Cryptococcus neoformans* isolates of this study, all of genotype VNI (columns 2 to 7; 9 and 10; 12 and 13). MM: 100 bp molecular marker (column 1). NC = negative control.

Table 1. Minimum inhibitory concentration (MIC), mean fractional inhibitory concentration index (FICI) and interactions of simvastatin (SIM) and amphotericin B (AMB) and fluconazole (FLZ) against environmental strains of *Cryptococcus neoformans*

Strain	Minimum inhibitory concentration (MIC)											
	AMB (µg/ml)	AMB + SIM (µg/ml)	SIM (µg/ml)	SIM + AMB (µg/ml)	FICI (AMB + SIM)	Interaction	FLZ (µg/ml)	FLZ + SIM (µg/ml)	SIM (µg/ml)	SIM + FLZ (µg/ml)	FICI (SIM + FLZ)	Interaction
Candida krusei ATCC 6258	1.0	N/A	> 128	N/A	N/A	N/A	32	N/A	> 128	N/A	N/A	N/A
PI1543	1.0	0.250	> 128	0.250	0.251	Synergism	8	64	> 128	> 128	9	Antagonism
PI1544	0.25	0.125	> 128	0.250	0.501	Indifferent	8	64	> 128	> 128	9	Antagonism
PI16167	0.5	0.125	> 128	0.250	0.251	Synergism	8	64	> 128	0.25	8	Antagonism
PI16180	1.0	0.500	> 128	0.250	0.501	Indifferent	4	32	> 128	> 128	9	Antagonism
PI16198	0.5	0.125	> 128	0.250	0.251	Synergism	8	64	> 128	> 128	9	Antagonism
PI16199	0.5	0.500	> 128	0.250	1.000	Indifferent	8	64	> 128	0.25	8	Antagonism
PI16200	0.5	0.250	> 128	0.250	0.501	Indifferent	8	64	> 128	> 128	9	Antagonism
PI16202	1.0	0.250	> 128	0.250	0.251	Synergism	8	8	> 128	1	1	Indifferent
PI17123	0.5	0.125	> 128	0.250	0.251	Synergism	8	64	> 128	> 128	9	Antagonism
PI17213	0.5	0.125	> 128	0.250	0.251	Synergism	8	64	> 128	> 128	9	Antagonism

N/A = not applicable; AMB = amphotericin B; SIM = simvastatin; FLZ = fluconazole; FICI = fractional inhibitory concentration index.

used and conditions of bird rearing may interfere with the isolation rates.[21]

Cryptococcus neoformans and *Cryptococcus gattii* have at least eight different molecular types that present variable geographical distribution within the eco-epidemiological context of the disease in Brazil and worldwide. The isolates of the present study were identified as the VNI genotype. This result was concordant with what has previously been found in Brazil and other parts of the world: in previous studies, the VNI genotype was detected in 95% of the environmental samples in which *Cryptococcus neoformans* was identified.[19] The predominance of the VNI genotype among the isolates recovered also enables better interpretation of the antifungal susceptibility test, given the consequent homogeneity of the population. On the other hand, it is well known that certain genotypes, such as VNIV, can frequently be recovered from regions such as around the Mediterranean basin.[23]

The susceptibility profile of *Cryptococcus* spp. relating to antifungals was evaluated in different countries between 1990 and 2004. The resistance to amphotericin B, 5-flucytosine and fluconazole that was detected was less than 1%. Isolates from North America presented MIC ≤ 8 μg/ml in relation to fluconazole, while in other geographical regions (Latin America and Africa), the strains showed susceptibility to fluconazole in 94% to 100% of the cases. Additionally, the isolates presented 99% susceptibility to amphotericin B, with MIC ≤ 1 μg/ml.[24]

There is increasing interest in evaluating the antifungal activity of antifungal drugs, especially in the field of combined therapy.[25] In addition, evidence demonstrating the potential use of statins for preventing and treating infections has been reported. Statins have been shown to attenuate the pathogenicity of microorganisms through modulating the signaling and other regulatory pathways that are involved in the infection.[26,27] The activity of statins against *Cryptococcus* and *Candida* species, with particular emphasis on simvastatin, used in isolation or in combination with classical antifungals such as amphotericin B and fluconazole, was previously described by Brilhante et al.[10] Chin et al.[9] demonstrated that the statin fluvastatin had fungicidal action against different species of *Candida*. Moreover, a synergistic effect was observed through combinations of fluvastatin with fluconazole and itraconazole, two commonly used azole compounds. With these combinations, both fluconazole and itraconazole exhibited potent activity against species of *Candida* and also against *Cryptococcus neoformans*.

The *in vitro* interactions of the effects of various statins, including simvastatin and various azole antifungals, against different opportunistic pathogenic fungi such as *Candida* and *Aspergillus* species, were investigated in another study. Fluconazole was found to act synergistically against *Aspergillus fumigatus* in combination with simvastatin, lovastatin and atorvastatin. The interaction of the combination of miconazole and simvastatin against *Candida glabrata* was not significant, but the sensitivities to this azole compound differed by one or two dilution steps between the isolates.[28]

The results from the present study demonstrated the efficacy of simvastatin as a synergistic agent in combination with amphotericin B against environmental isolates of *Cryptococcus neoformans*. However, no inhibition of yeast growth was observed when the effect of simvastatin was evaluated in isolation or in combination with fluconazole, and the MIC values found were elevated. Similar results were demonstrated by Brilhante et al.,[10] such that all the strains of *Cryptococcus neoformans* evaluated were inhibited by the combination of simvastatin and amphotericin B. However, no such inhibition was observed in relation to the combination of simvastatin and fluconazole.

The antifungal effect observed when simvastatin was evaluated in combination with amphotericin B may have been due to the action of statins in the process of formation of ergosterol, which has similarity to human cholesterol. This drug acts by inhibiting 3-hydroxy-3-methyl-glutaryl-coenzyme-A (HMG-CoA) reductase in both humans and fungi, since both of these act on the same synthetic pathway as mevalonate. The *in vitro* activity of statins (fluvastatin, simvastatin, pravastatin and lovastatin) against strains of species of *Candida* and *Cryptococcus* was evaluated, and only fluvastatin demonstrated inhibition of these yeasts. However, *in vitro* interaction of fluvastatin with fluconazole and amphotericin B has been observed, which demonstrates the potential synergism between these drugs.[9]

It is important to note that, in the present study, two isolates had a MIC of 1.0 μg/ml for amphotericin B, which was the highest MIC among the strains evaluated. These isolates presented a considerable reduction in MIC (0.250 μg/ml) when the combination of amphotericin B and simvastatin was tested, which again emphasizes the potential use of this combination of these drugs. On the other hand, among the four isolates presenting indifferent results regarding the association between amphotericin B and simvastatin, three of them demonstrated values that were slightly above the cutoff that had been adopted.

Given that all of the isolates of the present study belonged to the same molecular type, it is possible that a greater number of samples tested would have enabled clear demonstration of the synergistic effects between these two drugs. However, we collected the number of samples that were available at the time when the study was performed (i.e. 206 samples). Our isolation rate of 14.5% was in line with what had been found in other studies using the same methodology.[19,20] On the other hand, increasing the number of samples would also potentially have led to greater variability of the fungi in the samples (there are eight molecular types of *Cryptococcus*).

CONCLUSION

The data of this study demonstrate that simvastatin should be considered to be a possible therapeutic alternative, with the capacity to

potentiate the action of amphotericin B. Through using this drug, the duration of cryptococcosis treatment could potentially become shorter and, consequently, the time for which patients are exposed to the toxic effects of this antifungal could be reduced. In addition, statins may have an important role in the future, as a new treatment alternative in situations of resistance to antifungals.

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Accuracy of endocervical cytological tests in diagnosing preinvasive lesions of the cervical canal in patients with type 3 transformation zone: a retrospective observational study

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ABSTRACT

BACKGROUND: Cervical cancer screening in Brazil is done using Pap smears. Women who are most likely to have a preinvasive lesion or cervical cancer are immediately referred for colposcopy.

OBJECTIVE: The aim of this study was to evaluate the diagnostic performance of endocervical cytological tests in diagnosing preinvasive cervical lesions in women with initial high-grade squamous intraepithelial lesions (HSIL), or atypical squamous cells in which high-grade lesions could not be ruled out (ASC-H), or atypical glandular cells (AGC), and whose colposcopy did not show any abnormalities, with no fully visible transformation zone (types 2 and 3).

DESIGN AND SETTING: Retrospective observational study conducted in Rio de Janeiro, Brazil.

METHODS: Data from women who came to the cervical pathology outpatient clinic between January 2012 and April 2017 were analyzed. The results from endocervical cytological tests were compared with the final diagnosis, which was obtained through examination of a surgical specimen or, among women who did not undergo an excisional procedure, after cytological and colposcopic follow-up for two years.

RESULTS: We included 78 women. The sensitivity of endocervical cytological tests was 72.7%; specificity 98.5%; positive and negative predictive values 88.9% and 95.6%, respectively; and positive and negative likelihood ratios 48.7 and 0.28.

CONCLUSION: Endocervical cytological tests are simple, inexpensive and noninvasive, and form a reliable method for determining management among patients with HSIL, ASC-H and AGC cytological findings and negative colposcopic findings without visualization of the squamocolumnar junction.

INTRODUCTION

Cervical cancer is the fourth most common type of cancer among women worldwide, accounting for 570,000 new cases and 305,000 deaths in 2018.[1] In Brazil, 16,370 new cases were expected for that same year. In 2016, it was the third most frequent tumor, and the fourth largest cause of cancer-related death, excluding non-melanoma skin cancer.[2] While mortality has declined over recent years,[3] cervical cancer remains a public health problem, and reducing its incidence and mortality is still an ongoing challenge.

Periodic conventional Pap smears remain the most widely adopted strategy for cervical cancer screening in Brazil. When the results from this test show alterations that are most likely to represent the presence of cancer or its preinvasive lesions, immediate referral for colposcopy is necessary.[4] These lesions can include presence of atypical squamous cells in which high-grade lesions cannot be ruled out (ASC-H); atypical glandular cells (AGC); high-grade squamous intraepithelial lesions (HSIL); high-grade squamous intraepithelial lesions in which microinvasion cannot be ruled out; invasive squamous carcinoma; adenocarcinoma in situ (AIS); and invasive cancer.

When the squamocolumnar junction (SCJ) is not completely visible (type III transformation zone, TZ), colposcopy cannot rule out the presence of endocervical disease. In these cases, the most accurate method for doing so, or for treating it if present, is type III excision (cervical conization). However, this procedure increases the risk of obstetric complications[5] or other disorders, such as endocervical canal stenosis, thereby impairing these patients' follow-up,[6] and possibly compromising their quality of life.

Alternatively, endocervical cytological tests may aid diagnosis and management, often preventing conization, especially if colposcopy does not show any alteration. In Brazil, this test is recommended for women with the abovementioned cytopathological diagnoses, type III TZ and without colposcopic abnormal findings, but for whom there is a lack of definitive evidence.[4]

A systematic review of the literature using the terms "endocervical canal cytology", "endocervical canal", "endocervical canal cytology accuracy", in PubMed, SciELO (Scientific Electronic Library Online) and EMBASE (Excerpta Medica Database), found only one study evaluating the performance of endocervical cytological tests in a similar situation, albeit using a non-conventional processing method.[7]

A new search for papers in PubMed using the terms "endocervical brushing" and "accuracy" returned nine papers. The most recent of these evaluated cytology tests in a liquid medium or compared sample collection methods. However, none of these studies provided an adequate gold standard for estimating the accuracy of detection of preinvasive endocervical canal disease in the situation studied here, i.e. baseline cytological assessment of ASC-H, HSIL or AGC, with no fully visible transformation zone and no abnormal colposcopic findings.

The lack of evidence regarding endocervical cytological test performance in this situation causes considerable uncertainty regarding its usefulness in clinical practice for defining which women should undergo an excisional procedure and which of them just need to be kept under follow-up.

OBJECTIVE

The aim of this study was to establish the diagnostic performance of conventional endocervical cytological tests for diagnosing preinvasive and invasive cervical lesions in women with Pap smears showing ASC-H, HSIL or AGC without colposcopic abnormal findings and with the SCJ not fully visible (type III TZ).

METHODS

This was an observational study, in which information was obtained from our institution's cervical pathology outpatient database, complemented by data extracted from medical records.

Women who first came to our clinic between January 2012 (after the first publication of the Brazilian guidelines[8] recommending the use of endocervical cytological tests in these situations) and April 2017 were considered eligible. Data relating to the initial cytological tests, colposcopic evaluation, endocervical cytological test result and the final diagnosis were used.

The baseline cytological data were obtained conventionally at primary healthcare facilities of the Brazilian National Health System (Sistema Único de Saúde, SUS). At these facilities, cytological laboratories are subjected to internal and external quality monitoring, as recommended.[9]

Women with an indication for colposcopy are referred for diagnosis and treatment at units that can provide care for cases of higher complexity, such as the institution where the study was carried out. This institution has one of the referral units for diagnosing and treating preinvasive cervical lesions in the state of Rio de Janeiro.

Endocervical cytological specimens were obtained by means of endocervical brushing, which was done after the colposcopic examination. These specimens were processed using the conventional method (Papanicolaou).

Type III excision procedures (cervical conizations) were performed by means of electrosurgery, using a 2 cm loop electrode or a straight wire electrode to reach a depth of at least 2 cm in the endocervical canal.[10] The length of follow-up for women who did not undergo the excisional procedure was two years, with semiannual cytological tests and colposcopy. After this period, if there was no evidence of preinvasive disease or cancer, they were discharged for cytological follow-up at the primary healthcare facility. Any new tests with results suggesting preinvasive lesions or cancer would lead to an excisional procedure, as recommended in the current Brazilian guidelines.[4]

The colposcopies, excisional procedures, cytopathological tests and histopathological examinations were performed by the highly experienced colposcopists, cytologists and pathologists at Instituto Nacional de Saúde da Mulher, da Criança e do Adolescente Fernandes Figueira/Fundação Oswaldo Cruz (IFF/FIOCRUZ).

Study population

All the women who were referred to our institution from primary healthcare facilities and were received presenting either of the following were included in this study: (a) initial HSIL or ASC-H cytological and colposcopic findings without significant abnormalities, and with non-fully visible or partially visible SCJ (type III TZ); or (b) with initial AGC cytological findings, regardless of the type of TZ or abnormal colposcopic findings. In all these cases, the women underwent endocervical cytological tests to define the case management.

Patients whose endocervical tests done during the initial approach at our institution showed the same positive results as in the referral test, or more relevant results than those of the referral test, were considered positive. It was recommended that these patients should undergo an excisional procedure (type III excision, i.e. conization), although in reality not all of these patients underwent the procedure, while some others did so even though the new test had been negative. For patients who were considered negative (i.e. those with other results), it was recommended that they should be followed up for at least two years after the initial assessment.

Pregnant women were not included in this study because of the difficulty in following them up and the impossibility of performing an immediate excisional procedure. Women who were

lost to follow-up, despite repeated attempts to contact them via letters or by telephone, were excluded.

Diagnostic criteria

Endocervical cytological tests that showed diagnoses of HSIL, ASC-H or AGC were considered positive, since these results, according to the Brazilian Guidelines for Cervical Cancer Screening, indicate that an excisional procedure is needed in order to obtain the diagnosis. In cases in which squamous preinvasive disease is present, no further treatment is needed.[4] There were no cases with AIS in the endocervical cytological findings, or with cancer. All others, in which satisfactory material was obtained (including at least squamous and glandular or metaplastic epithelium) and did not show any of these cytological diagnoses, were considered negative.

Gold standard

The results from the endocervical cytological tests were compared with the definitive diagnosis that had been obtained through one of the following methods: (a) among women undergoing type III excision (conization), histopathological examination of the specimen obtained as part of the initial approach (through a positive endocervical cytological test); or (b) among women undergoing cytological and colposcopic follow-up (not undergoing excisional procedure as the initial approach), negative results from cytological and colposcopic examinations that were performed semiannually for two years or from histopathological examination of the specimen obtained through an excisional procedure performed after a new cytological or colposcopic examination showed a suspected preinvasive lesion during follow-up.

The final diagnosis took into consideration whether a preinvasive cervical lesion was present: either squamous (cervical intraepithelial neoplasia, CIN 2 or 3) or glandular (AIS). No cases of cancer were found. All other diagnoses were taken to be absence of preinvasive disease or cancer.

Data analysis

The data on the women included in this study were transferred to a spreadsheet. After checking for nonconformities, they were corrected and supplemented using information from the physical medical charts. Performance measurements were calculated from contingency tables that were constructed using the Statistical Package for the Social Sciences (SPSS), version 21.

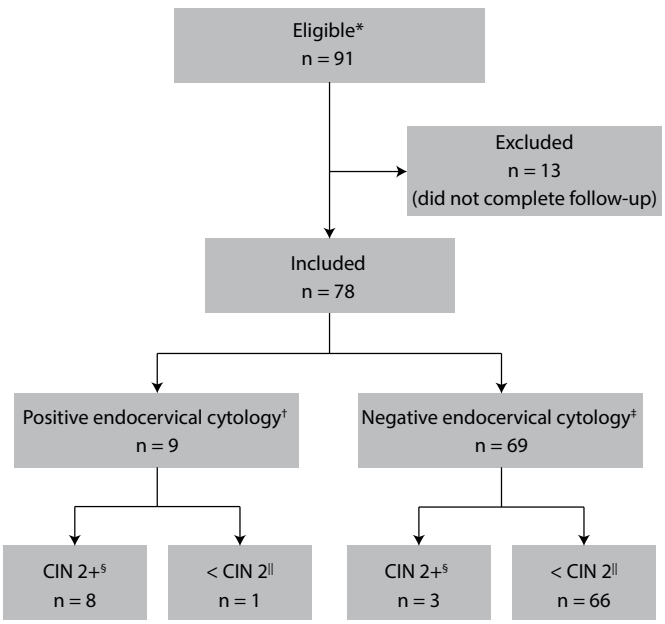
Ethical matters

This was a retrospective study. The stored data were identified only by the numbers of the medical records, thus preserving the women's identity. This study was approved by the local research ethics committee under the number CAE 03847218.0.0000.5269, on December 20, 2018.

RESULTS

Ninety-one women seen between January 2012 and April 2017 were found to be eligible. Of these, 13 were excluded because they had not completed a two-year follow-up. Hence, 78 patients remained in the study, among whom nine women were deemed to be positive, since they had endocervical cytological findings that maintained the cytological diagnosis of their referral or another result suggestive of preinvasive disease. These women underwent type III excision.

The remaining 69 women whose endocervical cytological findings suggested diagnoses of lesser importance than the initial one were deemed to be negative (**Figure 1**). Twelve women, out of the 78 women who remained in the study, underwent an excisional procedure after the early colposcopy (three despite negative endocervical cytological results) and the other 66 (66/78)



*Women who first came to our clinic between January 2012 and April 2017 presenting cytological findings of high-grade squamous intraepithelial lesion (HSIL), atypical squamous cells of undetermined significance in which high-grade lesions could not be ruled out (ASC-H) or atypical glandular cells of undetermined significance (AGC); colposcopic results without major abnormal findings or any findings suggestive of invasion; and not fully visible squamous-columnar junction (type III transformation zone, TZ) in the cases of squamous atypia, or, in the case of early AGC cytological findings, any type of TZ.

†Positive endocervical cytological findings = HSIL, ASC-H or AGC; ‡negative endocervical cytological findings = negative, low-grade intraepithelial lesion (LSIL) or atypical squamous cells of undetermined significance possibly non-neoplastic (ASC-US); §cervical intraepithelial neoplasia (CIN) 2+ = CIN II, CIN III or adenocarcinoma in situ (AIS); ‖< CIN 2 = CIN I or normal.

Figure 1. Study flowchart (IFF/FIOCRUZ, Rio de Janeiro, 2012-2017).

underwent semiannual cytological and colposcopic follow-up for two years. During this follow-up, only one patient had new positive cytological findings (AGC), at the six-month evaluation. This woman underwent an excisional procedure that confirmed the presence of preinvasive disease (CIN 3) and her case was considered to be a false negative from the endocervical cytological test that had been performed during the initial assessment. A total of 13 women underwent an excisional procedure (12 after the initial approach and one during follow-up). Among these, 11 had findings compatible with a preinvasive lesion (CIN 2-3 or CIN 2-3 with concomitant AIS) and two were negative (no abnormalities or CIN 1).

The characteristics of the women included, the initial colposcopic findings and the final diagnoses are shown in **Table 1**. Baseline cytological tests showing AGC were more frequent; the patients' mean age was close to 50 years; and HSIL was the preinvasive disease most often found among the women with baseline cytological tests.

Table 2 shows the results from the endocervical cytological tests and the gold standard that was established. It also shows the diagnostic performance measurements for all the women according to the nature of the cytological atypia (squamous or glandular) that were initially seen in these women. Some measurements could not be calculated due to lack of cases.

DISCUSSION

This study was conducted using retrospective data that were obtained from the clinical records of women who had been referred from primary care units in municipalities of the state of Rio de Janeiro, for diagnosis and treatment of preinvasive cervical lesions. All of the procedures performed were within our usual care. Our institution is one of the units that receive these women, and its practices follow the recommendations of the Brazilian Guidelines for Cervical Cancer Screening.[4]

The patients' mean age was 48.2 years. The low number of nulliparous women was as expected, given the inclusion criteria (especially regarding no visible SCJ). Because of the retrospective design of this study, little demographic information was available, but the characteristics of our sample resembled those of the regular users of public healthcare units in Rio de Janeiro.

Both the baseline cytological tests and the endocervical cytological tests done during colposcopy were performed by means of the conventional method that is predominantly used in laboratories that provide services to SUS. Accordingly, the results observed here apply to most Brazilian referral services that follow the same recommendations.

In our sample and our setting, endocervical cytological tests among women with type III TZ without abnormal colposcopic findings and with HSIL, ASC-H, and AGC cytological screening findings was most useful when this diagnosis was negative or when the results were less likely to represent a preinvasive lesion (ASC-US or LSIL). In these situations, the test ruled out disease in almost all healthy women, i.e. it diagnosed almost all true negatives. This can be inferred from the specificity of 98.5% and the negative predictive value (NPV) of 95.6% (**Table 2**).

Table 1. Characteristics of the women included, their initial examinations and final diagnoses (IFF/FIOCRUZ, Rio de Janeiro, 2012-2017)

Baseline cytological findings*	AGC n (%)	ASC-H n (%)	HSIL n (%)	Total
Mean age (SD)	35 (44.9) 45.4 (11.3)	27 (34.6) 52.1 (11.1)	16 (20.5) 47.7 (12.3)	78 48.2 (11.7)
Parity				
Nulliparous (%)	3 (8.6)	0 (0)	0 (0)	3 (7.3)
1-2 children (%)	7 (20.0)	11 (40.7)	1 (6.3)	19 (46.3)
3 or more children (%)	7 (20.0)	7 (25.9)	5 (31.3)	19 (46.3)
Unknown	18 (51.4)	9 (33.3)	10 (62.5)	37 (47.4)
Colposcopic findings				
Major abnormal† (%)	1 (2.9)	NA	NA	1 (1.3)
Minor abnormal (%)	9 (25.7)	8 (29.6)	1 (6.3)	18 (23.1)
Negative‡ (%)	25 (71.4)	19 (70.4)	15 (93.7)	59 (75.6)
Final diagnosis§				
CIN 2-3	1 (2.9)	3 (11.1)	6 (37.5)	10 (12.8)
CIN 2-3 + AIS	0 (0)	1 (3.7)	0 (0)	1 (1.3)
Negative	34 (97.1)	23 (85.2)	10 (62.5)	67 (85.9)

AGC = atypical glandular cells; ASC-H = atypical squamous cells in which high-grade squamous intraepithelial lesion cannot be ruled out; HSIL = high-grade squamous intraepithelial lesion; SD = standard deviation.

*Result from cytopathological examination that motivated the referral for colposcopy; †applies only to women with baseline cytological finding of AGC, since the inclusion criterion was absence of major findings in other women with cytological findings of ASC-H or HSIL (NA = not applicable); ‡includes normal, nonspecific and miscellaneous findings (endocervical polyps); §obtained through excisional procedure or through semiannual cytological and colposcopic follow-up for two years (positive = CIN 2-3 or CIN 2-3 + AIS [CIN 2 or 3 concomitant with adenocarcinoma in situ]; negative = CIN 1 or absence of intraepithelial disease).

The observed sensitivity of 72.7% also shows that endocervical cytological tests are also useful when positive, i.e. they show a high probability of being positive among women who truly have preinvasive disease. Although these tests do not detect all cases, they adequately indicate the excisional procedure most of the time, as expressed by the positive predictive value (PPV) of 88.9% (**Table 2**). It can also be considered that the excisional procedure was still useful when it did not show the presence of preinvasive lesions or cancer, since it served as a diagnostic method, thereby ensuring the absence of endocervical preinvasive disease in women who had had at least two positive cytological examinations (baseline and the one done during early colposcopy).

The large confidence intervals that were observed in the diagnostic performance measurements (**Table 2**) resulted from the small sample size. Despite the long study period of five years, few women met the requirements for using endocervical cytological findings as a predictor for endocervical disease.

The exclusion of women who had not completed two years of follow-up after negative endocervical cytology results contributed to this limitation. However, this also had the aim of preventing the bias that would result from their inclusion, since they would not have had enough time to show one of the outcomes.

We calculated the diagnostic performance measurements separately between women whose baseline cytological tests showed squamous atypia (ASC-H or HSIL) and those whose tests showed results of glandular nature (AGC), in order to differentiate diagnostic performance according to the nature of the atypia seen in the baseline cytological tests. The performance of the endocervical cytological tests among the women whose baseline cytological findings consisted of squamous atypia was like that obtained overall, among all the women included in this study. Regarding the situations in which the baseline cytological tests showed glandular atypia, it was only possible to calculate the specificity and the NPV, since there were no cases of positive endocervical cytological data collected during early colposcopy in this group. The measurements calculated also pointed towards excellent specificity and high NPV values. However, all of these measurements were less accurate because they were calculated among only part of the total sample.

Since the predictive values may vary with the prevalence of the disease in a specific population, these results can only be applied to services with the same patient profile as ours, i.e. those that only receive patients who are referred and approached in accordance with the current guidelines, from populations with the same disease prevalence, which is very difficult to ensure. Thus, we chose to calculate the positive likelihood ratio (LR+) and negative likelihood ratio (LR-), which are measurements that provide predictions of the presence or absence of disease, regardless of disease prevalence. In our study when the test was positive, LR+ was 48.7; and when negative (LR-), 0.28. These measurements can be considered to represent excellent performance, since they give rise to a significant change in the pretest probability of the presence of a preinvasive or invasive disease when the result from the endocervical cytological test is positive and exceeds the threshold for making the decision to undertake an excisional procedure. Conversely, a negative result provides a

Table 2. Distribution of endocervical cytological results, final diagnoses and diagnostic performance measurements regarding endocervical cytological testing (IFF/FIOCRUZ, Rio de Janeiro, 2012-2017)

Endocervical cytological findings	Preinvasive disease present*	Preinvasive disease absent*	Total	Sensitivity % (95% CI)	Specificity % (95% CI)	PPV % (95% CI)	NPV % (95% CI)	LR+	LR-
All cases				72.7 (46.4-99.0)	98.5 (95.6-100)	88.9 (68.4-100)	95.6 (90.8-100)	48.7	0.28
Positive†	8	1	9	-	-	-	-	-	-
Negative‡	3	66	69	-	-	-	-	-	-
Total	11	67	78	-	-	-	-	-	-
Squamous abnormality§				80.0 (55.2-100)	96.9 (91.1-100)	88.9 (68.4-100)	94.1 (86.2-100)	26.4	0.21
Positive†	8	1	9	-	-	-	-	-	-
Negative‡	2	32	34	-	-	-	-	-	-
Subtotal	10	33	43	-	-	-	-	-	-
Glandular abnormality‖				¶	100 (¶)	¶	97.1 (91.6-100)	-	-
Positive†	0	0	0	-	-	-	-	-	-
Negative‡	1	34	35	-	-	-	-	-	-
Subtotal	1	34	35	-	-	-	-	-	-

PPV = positive predictive value; NPV = negative predictive value; LR+ = positive likelihood ratio; LR– = negative likelihood ratio.
*Compounding of histopathological examinations on surgical specimens in women who underwent an excisional procedure or semiannual cytological and colposcopic follow-up for two years; †Cytological findings of high-grade squamous intraepithelial lesion (HSIL), atypical squamous cells in which high-grade squamous intraepithelial lesion (ASC-H) cannot be ruled out, or atypical glandular cells (AGC); ‡negative cytological findings or cytological findings of atypical cells of undetermined significance that are possibly non-neoplastic (ASC-US) or low-grade squamous intraepithelial lesion (LSIL); §baseline cytological and colposcopic findings of HSIL or ASC-H; ‖baseline cytological and colposcopic findings of AGC; ¶not possible to calculate.

safe possibility for follow-up. This safety was emphasized by the absence of invasive carcinoma in our sample and the possibility of detection of preinvasive disease during the follow-up, as occurred in one case in our study.

Negative predictors are essential because they reduce the risk of overtreatment, which could lead to reproductive problems in the future for these women (such as miscarriage and premature birth),[5] along with cervical canal stenosis. Such situations would impair the follow-up among these women or lead to secondary dysmenorrhea or even hematometra, thus requiring new surgical procedures.[6]

The mean age that was observed in our sample resulted from the inclusion criteria through which the women were selected. This characteristic did not represent bias, since these were the women who would benefit most from endocervical cytological testing, to whom these results can be applied, following the recommendations of the current Brazilian guidelines.[4]

The low frequency of preinvasive disease among women with baseline cytological findings of AGC (1/35; 2.8%), compared with the frequency observed among women with baseline cytological findings of ASC-H or HSIL (10/43; 23.2%), may have been related to the higher frequency of preinvasive squamous cervical lesions in general and the greater difficulty of making a cytological diagnosis of AGC.

Our study showed results that were close to those obtained by the only paper identified in the systematic review cited above. In the study thus identified, Goksedef et al.[11] compared the performance of endocervical brushing with that of cervical curettage. In their study, endocervical cytological tests showed slightly higher sensitivity than what we observed (83.3%), and similar specificity (96.5%). The difference in sensitivity may be explained in terms of random error due to the small sample size of both studies, in association with other variations, such as their inclusion criteria, since they only included women with LSIL as referral cytological results, among whom preinvasive lesions are known to present low prevalence. Moreover, those authors used a different brushing technique for sample collection: the material obtained through brushing was fixed in formaldehyde and processed for embedding in paraffin blocks, followed by histological analysis (rather than using conventional cytological analysis). This may produce performance differing from that of the usual technique. Hence, the results reported by Goksedef et al.[11] can only be extrapolated to sample collections using the same method. Apart from these factors, they did not define the method and depth of treatment excision that would be considered the gold standard in the study (which may have missed some positive results in the case of endocervical lesions) and did not find the diagnoses that were eventually obtained during the follow-up, when results that had been lost in the early evaluation could be obtained, as we did in our study, which decreased the sensitivity of the test in our research.

CONCLUSION

We can conclude that, in our practice, conventional endocervical brushing is a reliable test for determining the management that should be used among patients with HSIL, ASC-H and AGC screening cytological and colposcopic tests without abnormal findings and without a full view of SCJ (Type III TZ) as recommended in the Brazilian Guidelines for Cervical Cancer Screening.

The likelihood ratios found ensured that endocervical cytological testing presented good diagnostic performance, regardless of the prevalence of the disease among these women.

Further studies with larger sample sizes and in other scenarios are required to increase the accuracy and consistency of these results and to strengthen the recommendations for using this test to investigate women in the situation studied here.

Endocervical cytological testing is a simple low-cost noninvasive method that should be encouraged in referral centers that receive these patients. Its use can avoid unnecessary hospitalizations and invasive procedures, thereby reducing the potential complications of type III excision (conization).

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What are the perspectives for blood donations and blood component transfusion worldwide? A systematic review of time series studies

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Blood transfusion.
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Blood donation.
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Blood demand.
Time series.

ABSTRACT

BACKGROUND: Analysis of the literature suggests that changes relating to blood donations and blood component transfusion are occurring due to the aging of the population.

OBJECTIVE: To gain better understanding of the demand and supply of these inputs over time, and to identify the main associated demographic characteristics.

DESIGN AND SETTING: Systematic review conducted on time series relating to blood donations and blood component transfusions worldwide.

METHODS: A systematic review of the literature was conducted based on articles that presented time series relating to blood donation or blood component transfusion.

RESULTS: We found 1,814 articles. After the deletion process, only thirteen were read. Overall, these suggested that there is increasing demand for blood components and decreasing donation. The existence of seasonality regarding blood donation was pointed out. Men usually donated more blood and demanded more blood components than women. Approximately 50% of blood transfusions were performed in people aged ≥ 60 years.

CONCLUSIONS: This analysis on articles that presented time series relating to blood donations and blood component transfusion showed that aging of the population was the main factor associated with the increasing demand for blood and the decreasing supply of blood.

SYSTEMATIC REVIEW REGISTRATION: CRD42019118995.

INTRODUCTION

Because of the growing aging of the population, the demand for healthcare services, especially hospital services, is expected to increase.[1] At the same time, growing demand for blood components will accompany this increase.[2,3]

In addition to the costs and risks associated with the use of blood components, there has been much discussion in the literature regarding changes to the demand and supply of blood components, consequent to the aging of the population.[3-5]

Faced with the new projected demographic context, there are two healthcare problems: reduced blood donations and increased use of blood components, because the elderly population is the one that proportionally uses more of this input.[1-3,5-7]

Understanding the context and perspectives of blood supply and demand for blood components is essential, to make it possible to define strategies relating to these inputs.

OBJECTIVE

The objective of this study was to conduct a systematic review of the literature, with a search for studies on time series relating to blood donation rates and blood component transfusions, along with the demographic characteristics of the blood donor and blood recipient populations.

METHODS

This was a systematic review study that was conducted in accordance with the Preferred Reporting Items For Systematic Methodology in Meta-Analyses (PRISMA). The review protocol was previously registered with PROSPERO under the identification code: CRD42019118995.

To identify articles, a search on this topic was carried out in January 2019 in the national and international literature, for papers published between January 1, 2005, and December 31, 2018. Articles were selected through a four-stage process.

In the first stage, three bibliographic searches were performed. The first consisted of a search using the descriptors "blood donation" and "time series" in the SciELO, PubMed and Medline databases. The second consisted of a search using the descriptors "blood transfusion" and "time series" in the SciELO, PubMed and Medline databases. The third consisted of a search in the gray literature on this subject based on the literature that is considered classic for the subject and its references.

The second stage of article selection involved exclusion of texts that were found in duplicate.

The third stage of article selection involved exclusion of studies that did not present the research topic, either in the title or in the abstract.

Lastly, the fourth stage of article selection involved assessment of the eligibility of the texts that met the objectives of this review, i.e. studies that presented a time series for blood donation or blood component transfusion.

Results presentation

The results were presented in table form. To extract the data from the articles, an instrument for summarizing the main characteristics of the selected articles was elaborated. The data extracted were the following: author and year of publication; place of publication; type of outcome analyzed (transfusion or donation); and number of study participants (donors and/or patients).

Next, the main findings of the articles that were selected to form part of the time series were highlighted. These were the following: the observed trends (decrease or increase) for blood supply and blood component demand; and the observed seasonality of blood supply and blood component demand.

The demographic characteristics (gender and age) of the blood donor and blood component recipient populations presented in the selected articles were then explored. In this regard, we sought to answer two questions relating to blood supply: Who donates more blood: men or women? Young or old? Two questions relating to blood component transfusions were also answered: Who receives more blood components: men or women? Young or old?

Lastly, for each study, the factors associated with the outcomes that were found were identified, especially demographic and epidemiological factors.

Analysis of results

The studies were analyzed separately by two researchers and any discrepancies found were addressed by a third researcher. Initially, the main characteristics of the articles were presented, such as the representativeness of the countries and the journals with the largest number of articles published.

Next, the main outcomes found in the articles regarding the time series were analyzed. These were the trends and seasonality of blood supply and use of blood components. Lastly, the main demographic and epidemiological findings that explained the behavior of the series presented in each study were also analyzed.

RESULTS

Selection of texts

We found 241 articles (197 in PubMed, one in SciELO and 43 Medline) in the first search; 1,559 articles (809 in PubMed, 22 in SciELO and 728 in Medline) in the second search; and fourteen articles in the third search, thus totaling 1,814 articles gathered in the first stage of article selection.

The second stage of article selection involved exclusion of duplicate articles. At this stage, 720 articles were excluded, which left 1,094 articles for assessment of eligibility.

The third stage of article selection involved exclusion of articles that did not present the research topic in the title or abstract. In this phase, 1,072 articles were excluded, thus leaving 22 articles for assessment of eligibility.

In the fourth stage of article selection, the articles that met the review objectives were selected, i.e. articles that presented a time series for blood donation or blood component transfusion. At this stage, thirteen articles were selected and nine articles were excluded. The articles thus selected and the justifications for excluding articles at this stage are shown in **Figure 1**.

Features of articles

Regarding the general characteristics of the articles, the oldest dated from 2004, and the most recent from 2018. The journal with the largest number of articles published was Transfusion (seven articles). Germany was the place from which the largest number of articles was selected. The number of subjects in the studies ranged from around 70,000 to around four million. The length of follow-up of the time series ranged from one to 61 years.

Eight articles presented results relating to donation and ten articles presented results relating to blood component transfusions. Eleven articles presented results relating to trend and two, seasonality of the time series.

In total, 16 countries were represented in the thirteen articles included in this review. The identification and main characteristics of the articles are shown in **Table 1**.[8,20]

Outcomes

The most common temporal outcome regarding blood donations was reduced supply. With regard to seasonality, there were

significant reductions in blood donation over holiday periods or holiday weeks. Regarding demographic variables, men generally donated more blood than women, in all the articles that presented such a comparison.[9,11-17,20] People aged between 17 and 35 years were the ones who donated the most blood.

Regarding blood component transfusions, the most common temporal outcome was an increasing frequency of transfusions performed with increasing age of the population.[8,10,13-17,19,20] The series analyzed in this study did not show any seasonality component. In general, men received more blood components than women.[9,11-17,20] On the other hand, people aged 65 and over received the largest number of blood component transfusions.

The main outcomes relating to demographic characteristics and to blood donations and blood component transfusions are presented in **Table 2.**

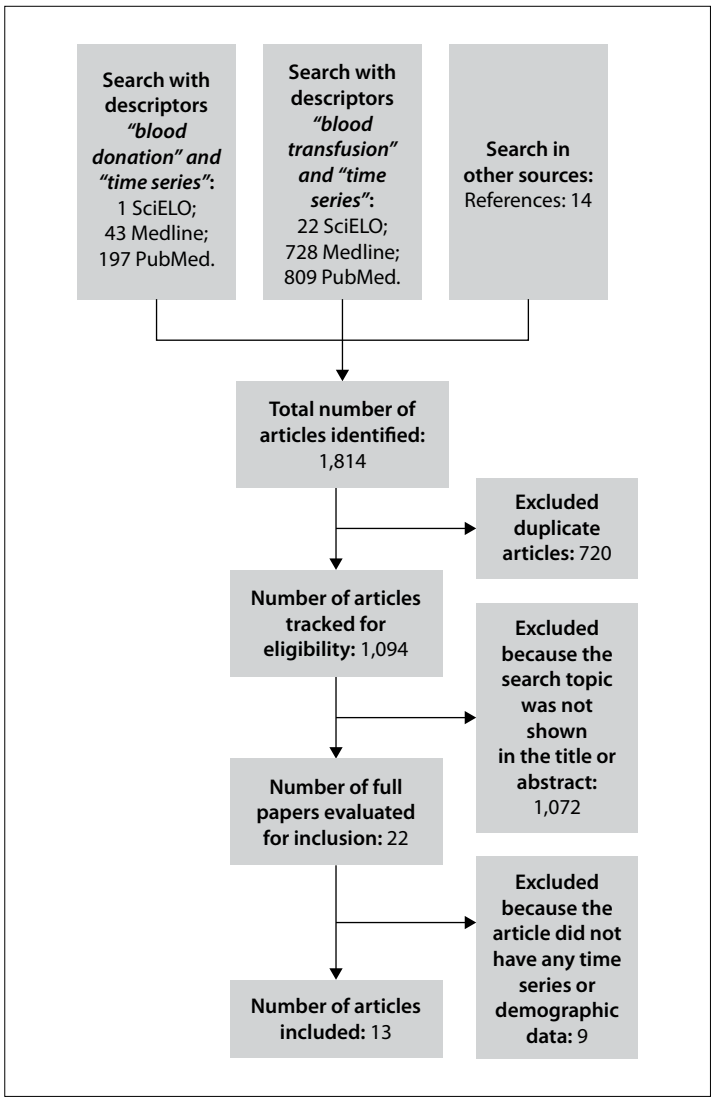

Figure 1. Flowchart for identifying and selecting articles for this systematic review.

Regarding the factors associated with the outcomes analyzed, ten articles presented possible epidemiological explanatory factors[8-10,12-14,16,17,19,20] and eleven presented possible demographic explanatory factors.[8-17,20] Increasing numbers of surgical procedures, greater circulatory system care and larger numbers of neoplasms were the main explanatory reasons found among the articles.

Population aging and migration were the main demographic factors associated with the outcomes. The main factors associated with the outcomes presented in the articles assessed here are organized in **Table 3**.

Based on this review of the literature on time series relating to blood donations and blood component transfusions, it was possible to identify population aging as the main factor responsible for the context of the growing demand for blood component transfusions and the drop in blood donations.

DISCUSSION

The main finding of this study was that increased demand for blood component transfusions was occurring simultaneously with reductions in blood donation rates. This temporal perspective points towards future blood shortages, due to the increasing size of the elderly population and the reduction in size of the population that is able to donate.

The presence of chronic diseases such as those relating to the circulatory system and neoplasms is inherent in elderly populations.

Table 1. Identification and characteristics of the articles

Article code	Author	Country	Outcome	Number of individuals
1	Ali et al.[8]	Finland	Transfusion	---
2	Borkent-Raven et al.[9]	Netherlands	Transfusion	290,043
3	Borkent-Raven et al.[10]	Netherlands	Donation/ Transfusion	---
4	Carneiro-Proietti.[11]	Brazil	Donation	426,142
5	Crawford et al.[12]	US	Donation	---
6	Currie et al.[13]	UK	Donation/ Transfusion	70,208
7	Drackley et al.[14]	Canada	Donation/ Transfusion	209,515
8	Greinacher et al.[15]	Germany	Donation/ Transfusion	---
9	Greinacher et al.[16]	Germany	Donation/ Transfusion	---
10	Greinacher et al.[17]	Germany	Transfusion	95,477
11	Oliveira et al.[18]	Brazil	Donation	1,246,462
12	Pfuntner et al.[19]	US	Transfusion	---
13	Volken[20]	Switzerland	Donation/ Transfusion	3,931,955

In addition, technological development allows for longer life, but at the cost of increased numbers of surgical procedures. Both of these epidemiological contexts are associated with higher demand for blood component transfusion, as addressed here.

On the other hand, the change in the age structure of the populations studied in the articles of this review, in which the growth of the elderly population and the consequent reduction of the young population were evident, will bring with it reductions of the donor population. First, because the population eligible for donation will decrease. Second, because within the donor population there will also be significant changes. Replacement donors are among the older members of this population and spontaneous donors among the younger.[14]

Soon, the group of replacement donors will no longer be able to donate. At the same time, due to the steadily declining birth rate, the young population will not be sufficient to meet the estimated high demand.

In view of this context, several studies in the literature have addressed the ways in which new blood donors are recruited, to identify improvements in the efficiency of techniques and strategies.[21,22] Stimuli towards increasing the replacement donor group have also been addressed.[23]

Table 2. Temporal components and demographic characteristics of blood donation and blood component transfusion series

Article code	Trend	Seasonality	Gender	Age
1	Increased demand and decreased offer	---	---	Older people demanded more than the other groups
2	---	---	Men demanded more than women	Older people demanded more than the other groups (≥ 65 years demanded 42.6% of transfusions)
3	Increased demand and decreased offer	---	---	Older people demanded more than the other groups (≥ 65 years demanded 59% of transfusions)
4	---	---	Men donated more than women (65.4% of donations were made by men)	Youth group donated more than other groups (group 25-35 years made 35.5% of donations; group ≥ 55 years made 2.9% of donations)
5	Decreased offer	Among young people, peak in April and lowest between Sep and Nov; among the elderly, peaks in January and summer	Men donated more than women (57.4% of donations were made by men)	Youth group donated more than other groups (18-24 years)
6	Increased demand and decreased offer	---	Men donated more than women; women demanded more than men (56% of transfusions were performed on women)	Older people demanded more than the other groups (≥ 70 years demanded 46% of transfusions)
7	Increased demand of greater magnitude than increased supply.	---	Men donated and demanded more than women	Youth group donated more than other groups (group 17-35 years)
8	Increased demand and decreased offer	---	Men donated and demanded more than women	Youth group donated more than other groups (group 20-29 years); older people demanded more than the other groups (≥ 60 years)
9	Increased demand and decreased offer	---	Men donated more than women	Youth group donated more than other groups; older people demanded more than the other groups (≥ 65 years)
10	Increased demand and decreased offer	---	Men donated and demanded more than women (52% and 54.2% respectively)	Youth group donated more than other groups (group between 20 and 44 years made 30.9% of donations); older people demanded more than the other groups (≥ 65 years required 47% of transfusions)
11	Stationary series	Lower in weeks with holidays	---	---
12	Increased demand and decreased offer	---	---	Older people demanded more than the other groups
13	Increased demand and decreased offer	---	Men donated and demanded more than women (57.3% of transfusions)	Youth group donated more than other groups (18-24 years); older people demanded more than the other groups (≥ 65 years required 48% of transfusions)

Before this, however, there is a need to focus on reducing the number of blood component transfusions. Alternative blood transfusion strategies that are safe, efficient and cost-effective have been considered in the literature. The use of these strategies is more frequent among organizations offering healthcare services. Bloodless medicine and surgery programs that seek to reduce blood use have significantly reduced the number of transfusions. These actions are implemented through protocol changes, incorporation of new technologies and more accurate control over blood component requests.[3-5,7,24-26]

Despite the large number of articles published in the databases that were searched in this study, few studies have addressed blood donation and transfusion rates from the perspective of statistical time series analysis.

In addition, the authors of the various studies presented their results differently, which makes it difficult to analyze the studies in an integrated manner. With regard to demographic variables, for example, there is a distinction between the countries from which the data were extracted. In Brazil, elderly people are defined as those aged 60 years and over. On the other hand, in Europe, elderly people are generally considered to be those aged 65 and over.

Table 3. Epidemiological and demographic factors associated with outcomes

Article Code	Epidemiological factors	Demographics
1	Increase in numbers of surgical procedures, procedures relating to the circulatory system and neoplasms	Ageing population
2	Increase in numbers of procedures relating to neoplasms and the circulatory system, pregnancy and childbirth	Ageing population
3	---	Ageing population
4	Number of donors women significantly lower than donors men, due to iron deficiency in population and neoplasms	Largest donations by replacement donors
5	Disasters and terrorist attacks significantly increase the number of donations	Ageing population
6	In the transfused group, 67% received an average of 2 transfusions while 33% received 5 or more transfusions	Ageing population
7	Transfusions in women are associated with pregnancy, childbirth and other events relating to the reproductive system	Ageing population
8	---	Ageing population; migration
9	Increase in number of surgical procedures relating to the circulatory system; family, pregnancy, iron deficiency and work were mainly responsible for non-donation among women	Ageing population
10	Increase in number of medical interventions	Ageing population
11	---	---
12	Increase in number of medical interventions	---
13	Increase in number of medical procedures and interventions relating to older people	Ageing population

CONCLUSION

Because of the estimated demographic context and the impossibility of changes to the age structure of populations, and because so few studies have evaluated time series relating to blood donations and blood component transfusions, further studies are essential. In particular, there is a need for studies addressing epidemiological, clinical and demographic characteristics. The present study makes a contribution towards more accurate perceptions of blood donations and blood component transfusions worldwide.

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Effect of prophylactic transcatheter arterial chemoembolization on hepatocellular carcinoma with microvascular invasion after R0 resection. A case-control study

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Carcinoma, hepatocellular.
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AUTHORS' KEY WORDS:
HCC.
TACE.
MVI.

ABSTRACT

BACKGROUND: Transcatheter arterial chemoembolization (TACE) is thought to prevent recurrence of hepatocellular carcinoma (HCC), but its efficacy is a matter of controversy.

OBJECTIVES: We investigated the effect of preventive TACE on the tumor, nodes, metastasis (TNM) classification in cases of stage II HCC (T2N0M0) after R0 resection.

DESIGN AND SETTING: Case-control study conducted in a tertiary-level public hospital.

METHODS: We analyzed recurrence rates and mortality rates over time for 250 consecutive cases of HCC in TNM classification cases of stage II HCC (T2N0M0) after R0 resection. These cases were divided into patients who underwent TACE (TACE+) and presented microvascular invasion (MVI+; n = 80); TACE+ but did not present MVI (MIV–; n = 100); MVI+ but did not undergo TACE (TACE–, n = 30); and TACE–/MVI– (n = 40).

RESULTS: MVI+ patients in the TACE+ group had significantly lower recurrence rates and mortality rates at one, two and three years than those in the TACE– group (all $P < 0.05$). Among MVI– patients, the TACE+ group did not have significantly lower recurrence rates and mortality rates at one, two and three years than the TACE– group (all $P > 0.05$). Regardless of whether TACE was performed or not, MVI– patients had significantly lower recurrence rates and mortality rates at two and three years after their procedures than did MVI+ patients (all $P < 0.05$).

CONCLUSION: Recurrence rates and mortality rates for MVI+ patients were significantly higher than for MVI– patients, beyond the first year after TACE. Postoperative adjuvant TACE may be beneficial for HCC patients with MVI.

INTRODUCTION

Hepatocellular carcinoma (HCC) is one of the most common malignancies in the world[1,2] and causes around 500,000 deaths every year.[3] Although hepatectomy and liver transplantation are considered to be curative therapies for HCC,[1] HCC often relapses after surgery. Transcatheter arterial chemoembolization (TACE) is thought to prevent recurrence, but its efficacy is a matter of controversy.[4]

OBJECTIVES

To analyze the effect of preventive TACE on recurrence rates and mortality rates among patients with the TNM classification of tumors of patients with stage II HCC (T2N0M0) of HCC who underwent R0 resection. Our hypothesis for this study was that TACE would be equally effective for HCC patients with or without MVI.

METHODS

Study design and ethics

In this case-control study, we analyzed recurrence rates and mortality rates among 250 consecutive cases of HCC with TNM classification stage II (T2N0M0) after R0 resection. We compared four groups of patients according to presence of microvascular invasion and use of TACE.

All the patients gave their informed consent to participation in this study. The study was approved by our institution's ethics committee on January 4, 2005, under the approval protocol number 2005006.

Patients

We followed up all the 250 consecutive patients with HCC who underwent R0 resection between January 2005 and December 2014, over a 36-month period after their surgeries. All of these patients were treated in the Department of Hepatobiliary Surgery, Guizhou Provincial People's Hospital. The inclusion criteria were as follows:

1. Age more than 16 years and less than 65 years;
2. Histopathological classification of high/medium differentiation;
3. TNM classification as stage II (T2N0M0) for HCC;
4. Treatment by means of extended resection of the tumor, with resection margins of 2 cm;
5. Liver function: Child-Pugh score of not more than 9 points.

The exclusion criteria were as follows:

1. Presence of other serious life-threatening diseases, such as severe coronary heart disease, another malignant tumor, etc.;
2. Evidence of liver abscess, abdominal infection, biliary fistula or intraperitoneal hemorrhage;
3. Pregnancy.

We divided the cohort into four groups: Group 1, who underwent TACE (TACE+) and presented microvascular invasion (MVI+; n = 80); Group 2, who were TACE+ but did not present MVI (MIV−; n = 100); Group 3, who were MVI+ but did not undergo TACE (TACE−, n = 30); and Group 4, who were TACE−/MVI− (n = 40).

TACE

Patients who underwent TACE did so within one to two months after their hepatectomies (**Table 1**). A hepatic arterial catheter was placed into the proper hepatic artery through the femoral artery using the Seldinger technique, and TACE was performed for the entire remnant liver. Hepatic angiography, computed tomography (CT) angiography, or both, were performed to detect any obvious tumor stains in the remnant liver.

The TACE procedure was a "sandwich" method, in which iodide oil (1 ml to 2 ml) was injected before and after administering chemotherapy. The chemotherapy regimen included fluorouracil, a platin (cisplatin or carboplatin) and adriamycin (doxorubicin or epirubicin). The dosages of fluorouracil, platin and adriamycin were determined according to body surface area and underlying liver function. All patients in this study who underwent prophylactic TACE received only one prophylactic TACE treatment, within two months after their surgery.

In order to make comparisons and avoid bias, we selected the cases with similar age (16 to 65 years), tumor differentiation (high/medium differentiation), tumor stage (T2N0M0) and Child-Pugh score for liver function (not more than nine points) and the cases with fewer complications (cases without liver abscess, abdominal infection, biliary fistula or intraperitoneal hemorrhage, etc.) and clean cutting edges (with resection margins of 2 cm). The aim of making this selection was to minimize other possible factors. We collected data mainly from the medical records (the period of time that was considered for data collection was from January 2005 to December 2017). In a very small number of cases, we collected data through patient follow-up.

Statistical analysis

We analyzed recurrence rates and mortality rates at one, two and three years after the procedures that were performed on these patients. Statistical analyses were performed using SPSS 16.0 for Windows (SPSS Inc., Chicago, IL, USA). The differences between groups of data were analyzed by means of the chi-square test (two-tailed). P-values < 0.05 were considered statistically significant.

RESULTS

During the study period, 280 patients were admitted to our hospital service presenting HCC, and 30 patients were excluded. The patients included comprised 131 males and 119 females. Their average age was 48.01 years (range: 16-65 years). The recurrence rates and mortality rates for each patient group, over each time period, are shown in **Tables 2, 3, 4 and 5**.

Tables 2 and 3 show that, among MVI+ patients, those who underwent TACE (TACE+ group) had significantly lower recurrence rates and mortality rates at one, two and three years after their procedures (all P < 0.05) than did those who did not undergo this procedure (TACE− group). The recurrence rates and mortality rates

Table 1. Demographic and clinical data of the four patient groups

	Group 1	Group 2	Group 3	Group 4	P
N	80 (M/F: 43/37)	100 (M/F: 51/49)	30 (M/F: 17/13)	40 (M/F: 21/19)	
Age (years)	48.62 ± 11.32	46.63 ± 11.61	45.45 ± 11.51	47.55 ± 11.55	> 0.05
Complications (%)	0.32 ± 0.13	0.35 ± 0.15	0.33 ± 0.12	0.34 ± 0.14	> 0.05
Liver function:					
Child-Pugh score	7.5 ± 1.4	7.3 ± 1.5	7.4 ± 1.3	7.4 ± 1.5	> 0.05

The P-values refer to the comparisons of age, complications and liver function Child-Pugh score in each group.

MVI = microvascular invasion; TACE = transcatheter arterial chemoembolization.

Group 1: underwent TACE and had MVI; Group 2: underwent TACE but did not have MVI; Group 3: had MVI but did not undergo TACE; Group 4: did not undergo TACE or have MVI.

among the MVI– patients tended to be lower at one, two and three years for the TACE+ group, but not significantly so (all P > 0.05).

Tables 4 and **5** show that, among both TACE+ and TACE– patients, those who were MVI– had significantly lower recurrence rates and mortality rates at two and three years than did those who were MVI+ (all P < 0.05).

DISCUSSION

Although preventive TACE has become a common post-surgical treatment for HCC,[4,5] its efficacy is still a matter of controversy. Support for TACE is based on the fact that compressing a tumor during surgery may lead to its spread. Postoperative TACE helps to clear up any proliferating, remnant or difficult-to-find tumor cells, and thus reduce early recurrence rates.[5,6]

A meta-analysis on four randomized controlled trials and three non-randomized controlled trials concluded that postoperative adjuvant TACE improves survival rates at two years and three years after resection.[7] The basis for opposing the use of TACE is that TACE can inhibit patients' immune systems, thereby contributing to tumor recurrence and metastasis.[8,9] Our results showed that among MVI– patients, TACE+ patients tended to have lower recurrence rates and mortality rates at one, two and three years, but not significantly so (P > 0.05), which indicates that preventive TACE cannot benefit MVI– patients.

Table 2. Recurrence rates among patients who did or did not undergo TACE

	Postoperative time	With TACE	Without TACE	P-value
With MVI	12 months	20/80 (25.0%)	14/30 (46.7%)	0.029
	24 months	35/80 (43.8%)	20/30 (66.7%)	0.032
	36 months	44/80 (55.0%)	23/30 (76.7%)	0.038
Without MVI	12 months	20/100 (20.0%)	9/40 (22.5%)	0.742
	24 months	29/100 (29.0%)	15/40 (37.5%)	0.328
	36 months	40/100 (40.0%)	21/40 (52.5%)	0.178

MVI = microvascular invasion; TACE = transcatheter arterial chemoembolization.

Table 3. Mortality rates among patients who did or did not undergo TACE

	Postoperative time	With TACE	Without TACE	P
With MVI	12 months	17/80 (21.2%)	13/30 (43.3%)	0.021
	24 months	32/80 (40.0%)	19/30 (63.3%)	0.029
	36 months	43/80 (53.8%)	23/30 (76.7%)	0.029
Without MVI	12 months	17/100 (17.0%)	7/40 (17.5%)	0.943
	24 months	26/100 (26.0%)	11/40 (27.5%)	0.856
	36 months	35/100 (35.0%)	18/40 (45.0%)	0.270

MVI = microvascular invasion; TACE = transcatheter arterial chemoembolization.

Table 4. Recurrence rates among patients who presented with or without MVI

	Postoperative time	With MVI	Without MVI	P
With TACE	12 months	20/80 (25%)	20/100 (20.0%)	0.423
	24 months	35/80 (43.8%)	29/100 (29.0%)	0.040
	36 months	44/80 (55.0%)	40/100 (40.0%)	0.000
Without TACE	12 months	14/30 (46.7%)	9/40 (22.5%)	0.033
	24 months	20/30 (66.7%)	15/40 (37.5%)	0.016
	36 months	23/30 (76.7%)	21/40 (52.5%)	0.038

MVI = microvascular invasion; TACE = transcatheter arterial chemoembolization.

Table 5. Mortality rates among patients who presented with or without MVI

	Postoperative time	With MVI	Without MVI	P
With TACE	12 months	17/80 (21.2%)	17/100 (17.0%)	0.469
	24 months	32/80 (40.0%)	26/100 (26.0%)	0.046
	36 months	43/80 (53.8%)	35/100 (35.0%)	0.012
Without TACE	12 months	13/30 (43.3%)	7/40 (17.5%)	0.018
	24 months	19/30 (63.3%)	11/40 (27.5%)	0.003
	36 months	23/30 (76.7%)	18/40 (45.0%)	0.008

MVI = microvascular invasion; TACE = transcatheter arterial chemoembolization.

The Milan criteria classify MVI as an independent risk factor for HCC,[10] and its presence in the hepatic or portal veins or the bile duct is an accurate predictor of recurrence risk and overall survival in patients with HCC after R0 liver resection and transplantation.[11-14] Postoperative adjuvant TACE may be beneficial for HCC patients with MVI.[15]

CONCLUSIONS

The recurrence and mortality rates among MVI+ patients were significantly higher than those of MVI– patients, beyond the first year after TACE ($P < 0.05$).

Thus, MVI+ patients may benefit from timely administration of postoperative adjuvant TACE if this is done within one to two months after R0 resection of HCC.

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Immunohistochemical assessment of symptomatic postmenopausal endometrial polyps in tamoxifen users and nonusers: a case control study

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ABSTRACT

BACKGROUND: Endometrial polyps are common in postmenopausal women, and the effect of tamoxifen use (a risk factor for endometrial polyps) on their pathogenesis is unclear.

OBJECTIVES: To evaluate the expression of hormone receptors and markers for proliferation/apoptosis (Ki-67 and Bcl-2) in endometrial polyps in postmenopausal users and nonusers of tamoxifen.

DESIGN AND SETTING: Cross-sectional analytical study in a tertiary-level academic hospital.

METHODS: 46 women (14 tamoxifen users and 32 nonusers) with postmenopausal bleeding underwent hysteroscopic resection of endometrial polyps. Polyp samples were immunohistochemically assessed for detection of Ki-67, Bcl-2 and estrogen and progesterone receptors.

RESULTS: Analysis on the glandular component of the polyps revealed progesterone receptor expression in the polyps of 96.9% of the nonusers of tamoxifen, and 92.3% of the tamoxifen users (P = 0.499). All polyps in nonusers and 92.3% of those in users were also positive for estrogen receptors (P = 0.295). Ki-67 was expressed in 75% of the polyps in the tamoxifen users and 82.8% of those in the nonusers. All endometrial polyps expressed Bcl-2.

CONCLUSIONS: The immunohistochemical analysis on endometrial polyps demonstrated that, although tamoxifen is considered to be a risk factor for endometrial polyps, there were no significant differences in the expression of hormone receptors between users and nonusers of tamoxifen. There were no between-group differences in Ki-67 and Bcl-2 expression, and all patients displayed inhibition of apoptosis by Bcl-2, thus supporting the theory that polyps develop due to inhibition of apoptosis, and not through cell proliferation.

INTRODUCTION

Even though endometrial polyps are common in postmenopausal women, their pathogenesis is still not entirely understood.[1] Polyps are most commonly diagnosed in the fifth decade of life,[2] and occur in 16 to 54% of women with postmenopausal bleeding.[3,4]

Late menopause, hormone therapy and tamoxifen use are considered to be risk factors for endometrial polyps. Although the etiopathogenesis of these polyps is still unknown, their presence is considered to be a risk factor for endometrial cancer. Tamoxifen users have higher incidence of polyps, possibly due to the estrogenic effects of tamoxifen on the endometrial epithelium.[5]

OBJECTIVE

Since there is no consensus in the literature regarding the pathogenesis of postmenopausal endometrial polyps, or regarding the effect of tamoxifen on the hormone receptor profile of these polyps, the aim of the present study was to investigate these issues. It was also sought to elucidate the effect of tamoxifen on markers for cell proliferation and apoptosis in postmenopausal polyps, given that the data in the literature are not consistent on this point.

METHODS

Patients

A cross-sectional analytical study was conducted on symptomatic postmenopausal women who were referred to the gynecological endoscopy service of the Santa Casa Hospital Complex

for hysteroscopic examination between January 2000 and December 2003, and who underwent surgical hysteroscopy for polypectomy. These patients were recruited through convenience sampling and were classified into two groups according to their use of tamoxifen: group 1, nonusers (controls); and group 2, users (cases).

A total of 46 postmenopausal women aged between 47 and 86 years participated in the study. All of the patients presented postmenopausal bleeding and underwent hysteroscopic resection of endometrial polyps.

Fourteen patients had been using tamoxifen for at least one year to treat breast cancer (group 2), while the remaining patients had never used hormone medication (group 1). The length of time since the menopause ranged from one to 35 years.

The polyp samples were cut and mounted on slides (total of 184 slides). However, three slides containing estrogen and progesterone receptors, thirteen Ki-67 slides and four Bcl-2 slides were discarded due to difficulties in slide preparation and reading.

Ethical considerations

This study was approved by our institution's research ethics committee (institutional review board-equivalent) under the number 251.296, on April 22, 2013. The study was conducted in accordance with the provisions of the Declaration of Helsinki.

Method

The immunohistochemical analysis was conducted using formalin-fixed and paraffin-embedded tumor tissue. The samples were then sectioned into slices of thickness 3 μm, deparaffinized and rehydrated prior to analysis. The Advance™ HPR kit (DakoCytomation®) was used for detecting Ki-67, Bcl-2 and estrogen and progesterone receptors.

Sodium citrate (pH 6.0) was used for Ki-67 antigen retrieval. Antigenic retrieval of Bcl-2, estrogen and progesterone was conducted using Tris-ethylenediaminetetraacetic acid (EDTA) (pH 9.0), through incubation at 95-98 °C in a water bath for 40 minutes. Endogenous peroxidase activity was blocked through incubation in 5% hydrogen peroxide (H_2O_2) 30V in methanol for two ten-minute periods. Nonspecific proteins were blocked using 1% bovine serum albumin for 30 minutes.

The slides were incubated with primary antibodies overnight at 4 °C. They were also subjected to incubation with secondary and tertiary antibodies for 40 minutes at room temperature. Samples of lymphoid tissue (tonsil samples) were used as a positive control for Ki-67 and Bcl-2, and mammary tissue was used as a control for the analysis on estrogen and progesterone. The same tissues were also incubated with all but the primary antibodies, which were replaced with 1% bovine serum albumin, as a form of negative control.

Antibody-antigen binding was viewed using diaminobenzidine chromogen. Harris hematoxylin was used for counterstaining, and the slides were dehydrated and mounted with synthetic resin.

All the immunostained slides for each antibody were analyzed and quantified separately by two observers using an optical microscope. Immunopositivity for estrogen, progesterone, Ki-67 and Bcl-2 was investigated in at least 10 high-power fields, and the results were considered based on immunoreactivity in the glandular epithelium.

Cases were considered positive for estrogen and progesterone receptors when immunostaining was observed in at least 1% of the gland cells in the sample, and were considered negative when immunostaining was not observed, or occurred in less than 1% of the sample.

The analysis on Bcl-2 was conducted through cytoplasmic immunostaining of gland cells. Absence of staining in the endometrial gland was indicative of a negative result, and reactions in at least one endometrial gland were considered to be positive results.

Ki-67 immunoreactivity was semi-quantitatively analyzed through nuclear immunostaining in the glandular component in the samples. Immunostaining in at least 5% of gland cell nuclei was considered indicative of high proliferation. Cases with an absence of immunostaining or immunoreactivity in less than 5% of cells were suggestive of low proliferation indices.

Statistical analysis

Qualitative variables were described as absolute and relative frequencies, and quantitative variables as the mean and standard deviation or the median and interquartile range.

Between-group comparisons of mean patient age and menopausal age were conducted using a t-test. Fisher's exact test was used to investigate associations between the presence or absence of hormone receptors (estrogen and progesterone) and markers for proliferation and apoptosis (Ki-67 and Bcl-2). The Mann-Whitney nonparametric test was used for between-group comparisons of the length of time since menopause and the presence or absence of progesterone receptors.

The analyses were performed using the Statistical Package for the Social Sciences (SPSS) software, version 17.0, and a 5% significance level was used.

RESULTS

The results regarding the expression of estrogen and progesterone receptors and proliferation (Ki-67) and apoptosis (Bcl-2) markers in the endometrial polyps of postmenopausal users (group 2) and nonusers of tamoxifen (group 1) are displayed in **Table 1**.

Estrogen and progesterone receptors were identified in the glandular tissue of endometrial polyps in both groups, and no between-group differences were found in relation to this variable.

The mean age of the patients in group 1 was 60.91 years, while the mean age in group 2 was 63.87 years, with no significant difference between the groups (P = 0.360).

As shown in **Table 2**, the mean age at which menopause occurred did not differ between the users (M = 49.29 years) and nonusers (M = 49.90) of tamoxifen (P = 0.727). Analysis on the length of time since the menopause and hormone receptor expression showed that the length of time since the menopause was significantly greater in the absence (median of 29 years) than in the presence (median of 9 years) of progesterone receptors, as shown in **Table 3** (P = 0.026).

All the patients had initially reported experiencing postmenopausal bleeding, and after ultrasound findings suggestive of endometrial polyps, all of them underwent hysteroscopy. The median length of time since the menopause at the time of hysteroscopic resection of endometrial polyps was 10.50 years (interquartile range 7.00-21.75) among users of tamoxifen and 8.00 years (interquartile range 3.76-14.00) among nonusers (P = 0.161). An inverse correlation was identified between the length of time since the menopause and the expression of progesterone receptors, as displayed in **Table 3** (P = 0.026).

After hysteroscopic resection, the polyps were classified as hyperplastic or atrophic. Atypical hyperplasia and cancer were not observed in any of the cases examined.

Table 1. Prevalence of hormone receptors (ER and PR) and markers for proliferation and apoptosis (Ki-67 and Bcl-2), according to tamoxifen use

Positive	Tamoxifen users	Tamoxifen nonusers	P
	Group 1	Group 2	
PR	31 (96.9%)	12 (92.3%)	0.499
ER	31 (100.0%)	12 (92.3%)	0.295
KI-67	24 (82.8%)	9 (75.0%)	0.627
Bcl-2	30 (100.0)	12 (100.0)	-

PR = progesterone receptor; ER = estrogen receptor.

Table 2. Sample characteristics

	Tamoxifen users	Tamoxifen nonusers	P
	Group 1	Group 2	
Age	60.91 ± 10.08	63.86 ± 9.66	0.360
Age at menopause	49.90 ± 4.02	49.29 ± 5.86	0.727
Length of time since menopause (years)	8.00 (3.75-14.00)	10.50 (7.00-21.75)	0.161

Mean ± standard deviation; median (P25-P75).

Table 3. Median and interquartile range of length of time since the menopause, according to presence or absence of progesterone receptors

	Progesterone receptors		P
	Positive	Negative	
Length of time since menopause	9.00 (3.50-12.50)	28.00 (12.25-34.00)	0.026

DISCUSSION

In the present sample, the similarities in mean patient age and age at the menopause between the groups were indicative of a homogenous and representative sample.

The pathogenesis of endometrial polyps is still not well understood, and there is no consensus regarding the treatment of postmenopausal polyps. Biron-Shental et al.[6] identified endometrial polyps in 25.9% of a sample of postmenopausal breast cancer patients who used tamoxifen. It has been reported that polyps become malignant in 3-10.7% of the cases.[7-9]

The role of estrogen in the development of endometrial polyps can be explored by assessing hormone receptors. Studies have shown that a reduced number of progestogen receptors (compared with estrogen receptors) are present in the stromal but not the glandular component of polyps.[10,11] In the present sample, glandular expression of progesterone receptors was found in 92.3% of the users of tamoxifen and 96.9% of the nonusers (P = 0.499). A total of 92.3% of the polyps in the tamoxifen users were estrogen-positive, while all the polyps of the nonusers displayed estrogen (P = 0.295).

These results demonstrate that, in the present sample, tamoxifen use did not significantly influence the expression of progesterone and estrogen receptors in postmenopausal endometrial polyps. Endometrial polyps may develop due to increased expression of estrogen receptors, reduced expression of progesterone receptors, or both.[10] In the present sample, no significant difference between the expressions of estrogen and progesterone receptors was found in endometrial polyps.

Taylor et al.[11] found that the increased expression of estrogen in polyps was limited to the glandular epithelium. Although the analyses in the present study were limited to the glandular epithelium, the findings are in agreement with those of Taylor et al.[11]

Studies on the pathogenesis of endometrial polyps have suggested that an imbalance between estrogen and progesterone may play a role in polyp growth.[3,10-15] Some studies have demonstrated decreased expression and others increased expression of hormone receptors in polyps.

Almeida et al.[3] assessed hormone receptors in endometrial polyps and the surrounding endometrium in postmenopausal women and found higher expression of estrogen and progesterone receptors in the polyps. They therefore suggested that low estrogen levels and endometrial atrophy might contribute to polyp development. They concluded that hormone receptors, especially estrogen, played an important role in the physiopathology of postmenopausal endometrial polyps.

Studies such as those by Mittal et al.[10] and Belisario et al.[16] have suggested that, in the absence of high estrogen levels, increased expression of estrogen and progesterone receptors in gland cells may contribute to polyp development. These results suggest that

the expression of estrogen and progesterone receptors differs between the stromal and glandular endometrium in postmenopausal patients.

Koshiyama et al.[12] studied 33 menopausal patients and found that there was a reduction in estrogen level with increasing age. In the present sample, the reductions in the numbers of hormone receptors were also proportional to the length of time since the menopause (P = 0.026).

It was found in the present study that the polyps in 75% of the tamoxifen users and 82.8% of the nonusers expressed Ki-67. The present results are in agreement with the data in the current literature, in that no between-group differences were found regarding Ki-67 expression.[17]

During the normal menstrual cycle, endometrial apoptosis occurs in the middle and at the end of the secretory phase, and during the first two days of menstruation. The balance between mitotic activity and apoptosis regulates endometrial development during the menstrual cycle.[18] The expression of Bcl-2 genes is inversely proportional to the level of apoptosis in the tissue. In the present sample, Bcl-2 was expressed in the endometrial polyps of all patients (users and nonusers of tamoxifen).

Morsi et al.[19] identified significant Bcl-2 expression in the glandular component of the endometrium in postmenopausal women, despite minimal expression in the endometrial stroma. Some studies have suggested that endometrial polyp growth occurs due to decreased apoptosis, characterized by increased Bcl-2 expression.[11,20]

One potential source of bias in the present study was that the participants were recruited through convenience sampling among women living in southern Brazil who presented symptomatic postmenopausal bleeding and were referred to a tertiary medical center for investigation. The best information regarding patients who underwent hysteroscopy was found to be over the period from 2000 to 2003. The sample of consecutive patients was selected according to the patients' records, and then immunohistochemical evaluation was performed. No computer system was used to analyze the immunohistochemical technique.

Despite these limitations, the present study provides an analysis on several consecutive cases and controls from a specific population of postmenopausal women who were either users or nonusers of tamoxifen and presented symptomatic endometrial polyps that were resected and immunohistochemically assessed. It is essential to emphasize the relevance of conducting immunohistochemical studies among patients who are using tamoxifen and of ascertaining the pathogenesis of the endometrial disease in this specific population, along with the role of hormone receptors in the pathogenesis of endometrial polyps. Although the study sample was small, this is a very important subject. Therefore, we would encourage further studies with the aim of clarifying the study questions.

CONCLUSIONS

Longer time since the menopause was associated with decreased expression of hormone receptors in endometrial polyps. Immunohistochemical analyses showed that, although tamoxifen use is generally considered a risk factor for developing endometrial polyps, hormone receptor expression did not differ between users and nonusers of tamoxifen. No between-group differences in Ki-67 and Bcl-2 expression were observed, and inhibition of apoptosis through Bcl-2 overexpression was observed in all participants. These findings support the theory that polyp growth occurs due to inhibition of apoptosis, rather than because of cell proliferation alone.

In the functional endometrium, apoptosis is related to hormone receptor expression and varies over the menstrual cycle. In postmenopausal women, apoptosis does not appear to be regulated by estrogen and progesterone.

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Comparison of scores for the classification of cardiometabolic risk in adult patients enrolled in a Venezuelan program for chronic non-communicable diseases: a cross-sectional study

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ABSTRACT

BACKGROUND: Several continuous measurements of cardiometabolic risk (CMR) have emerged as indexes or scores. To our knowledge, there are no published data on its application and validation in Latin America.

OBJECTIVE: To evaluate four continuous measurements of metabolic status and CMR. We established its predictive capacity for four conditions associated with CMR.

DESIGN AND SETTING: Cross-sectional study conducted at a healthcare center in the state of Carabobo, Venezuela.

METHODS: The sample comprised 176 Venezuelan adults enrolled in a chronic disease care program. Four CMR scores were calculated: metabolic syndrome (MetS) Z-score; cardiometabolic index (ICMet); simple method for quantifying MetS (siMS) score; and siMS risk score. CMR biomarkers, proinflammatory status and glomerular function were assessed. MetS was established in accordance with a harmonized definition.

RESULTS: Patients with MetS showed higher levels of all scores. All scores increased as the number of MetS components rose. The scores showed significant correlations with most CMR biomarkers, inflammation and glomerular function after adjusting for age and sex. In the entire sample, MetS Z-score, siMS score and siMS risk score showed the ability to detect MetS, reduced glycemic control, proinflammatory status and decreased estimated glomerular filtration rate. ICMet only discriminated MetS and proinflammatory state. There were some differences in the predictive capacity of the scores according to sex.

CONCLUSIONS: The findings support the use of the scores assessed here. Follow-up studies should evaluate the predictive capacity of scores for cardiovascular events and diabetes in the Venezuelan population.

INTRODUCTION

Cardiometabolic diseases are responsible for a significant number of deaths around the world.[1] In the year 2013, heart disease, diabetes mellitus and cerebrovascular diseases ranked as first, third and fourth leading causes of mortality in Venezuela.[2]

The likelihood of developing cardiovascular disease and diabetes mellitus is known as cardiometabolic risk. The etiopathogenesis of these diseases is complex and involves a wide range of interconnected cardiometabolic risk factors that often match in the same patient. The term metabolic syndrome describes the confluence of cardiometabolic risk factors in an individual, such as abdominal obesity, atherogenic dyslipidemia, hypertension, glucose intolerance/insulin resistance, microalbuminuria, proinflammatory and prothrombotic state. Presence of metabolic syndrome raises the cardiometabolic risk because this syndrome confers a fivefold increase in the risk of diabetes mellitus type 2 and a twofold increase in the risk of cardiovascular disease over the next five to ten years.[3]

There are various diagnostic criteria for metabolic syndrome, but in all cases, the diagnosis ends up using a cutoff point for a dichotomous response: absence or presence of metabolic syndrome when at least three of the five individual components of metabolic syndrome are accumulated. However, the expression of each risk component is continuous, and cardiometabolic risk is a progressive function of these combined risk measurements.[4,5,6] Use of dichotomous definitions for metabolic syndrome denies the scientific evidence, since it is a gradual condition in which the risk spectrum increases progressively with the number of anomalies or individual

factors accumulated.[7,8] Also, the conventional diagnosis of metabolic syndrome does not make it possible to follow up the gradual changes that occur in individuals with metabolic syndrome once the therapeutic measures are in place.[8]

A continuous cardiometabolic risk index responds to the above limitations. It shows the continuous risk to which an individual is exposed and provides information about the severity of the risk. Over recent years, several continuous measurements of cardiometabolic risk have emerged as indexes or scores. In general, these include the same individual components of metabolic syndrome but differ in the methodologies that are used for their construction and calculation.

None of these proposed continuous scores for metabolic syndrome originated from the Latin American population. To our knowledge, there are no published data on application and validation of such scores in Latin America. It is important to consider that the prevalence of this disease, its survival rates and the distribution of risk factors and their weights as determinants of the disease may be different in each population.[9] There is also genetic and environmental control over the expression of cardiometabolic risk factors in each population group.

OBJECTIVE

The aim of this research was to evaluate four continuous measurements of metabolic status and cardiometabolic risk in a group of adult patients who had been enrolled in the CAREMT (Cardio Renal Endocrine Metabolic and Tobacco) program, which was developed at a healthcare center in the state of Carabobo, Venezuela. We explored the variation of continuous measurements according to different biomarkers for cardiometabolic risk, inflammation and glomerular function. We established the ability of continuous measurements to discriminate or detect metabolic syndrome, reduced metabolic control, proinflammatory status and decreased glomerular function. This exploratory assessment was the first step towards validation of continuous measurements of cardiometabolic risk in Latin American countries such as Venezuela, for future primary care applications.

METHODS

Participants and data collection procedure

This was a cross-sectional study of correlational type, with a non-experimental design. The validation of continuous measurements was performed using a cross-section of baseline data from the CAREMT program, implemented at a primary healthcare center in the state of Carabobo, Venezuela. This program consists of an integration of the cardiovascular, endocrine, metabolic, renal, cancer and anti-smoking programs, in a strategy for screening and prevention of the most frequent non-communicable chronic diseases and their risk factors.

The Research Ethics Committee of the University of Carabobo approved this study (CPBBUC-002-2019-DIC-NR; code: KE94KD90; date: May 9, 2019). The study procedures followed the ethical standards of the Helsinki Declaration and its revisions. Informed consent was obtained from each participant.

The study was based on non-probabilistic sampling. The population comprised all the adult patients (20-65 years of age) of both genders who were enrolled in the CAREMT program between 2015 and August 2017 (n = 210). The sample was composed of 176 patients, after exclusion of patients with one or more of the following conditions: personal antecedents of cardiovascular or cerebrovascular events; body mass index under 18.5 kg/m² or greater than 35 kg/m²; significant alterations in muscle mass (amputations, loss of muscle mass, muscle diseases or paralysis); renal failure; pregnancy; lactation; severe hepatopathy; generalized edema; ascites; or incomplete anthropometric measurements or biochemical determinations.

We applied an instrument for collecting personal and biomedical data. The same interviewer always performed the interview to ensure standardization of the procedure. The participants underwent anthropometric-clinical measurements and a blood sample was taken. They were instructed to have a light dinner and to fast for 12 hours before blood collection. A partial morning urine sample was requested on the day when blood was collected.

Anthropometric, blood pressure and biochemical measurements

Weight and height measurements were made following standard protocols. Waist circumference was measured with a measuring tape at the midpoint between the last rib and the iliac crest, with the subject standing. This measurement was made at the end of an unstressed expiration. The waist circumference, body mass index (BMI; in kg/m²) and waist-to-height ratio (WHR; in cm/cm) were classified as elevated in accordance with the accepted criteria.[10,11,12]

Blood pressure was measured using a sphygmomanometer (Omron model M7; Omron Health Care, Kyoto, Japan). The diagnosis of arterial hypertension was established in accordance with international recommendations.[13] The percentage of body fat (%BF) was ascertained using a body composition analyzer (model TBF 300 A; Tanita, Tokyo, Japan). %BF ≥ 25% (men) and ≥ 30% (women) was considered elevated.[14]

The $A1_C$ hemoglobin fraction ($HbA1_C$) in whole blood was assessed by means of an immunoassay. Glucose, creatinine, triglycerides (TGL), total cholesterol (TC), low-density lipoprotein-cholesterol (LDLc) and high-density lipoprotein-cholesterol (HDLc) were determined in serum using colorimetric enzymatic methods. Serum high-sensitivity C-reactive protein (hsCRP) was quantified by means of immunoturbidimetry. The protein content in the partial urine sample was determined using a reactive tape.

Detection of a protein level of at least one cross (+) in the urine was defined as proteinuria.

TC/HDLc, LDLc/HDLc and TGL/HDLc ratios and the non-HDL cholesterol concentration (TC-HDLc) were calculated. The estimated glomerular filtration rate (eGFR) was obtained through the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation,[15] using the renal function calculator of the Spanish Society of Nephrology.[16]

Presence of metabolic syndrome and its individual components were established in accordance with a harmonized definition.[11] Presence of diabetes was defined using the criterion of the American Diabetes Association.[17] TC, LDLc, TC/HDLc ratio, LDLc/HDLc ratio and non-HDL cholesterol were classified as elevated in accordance with previously described criteria.[18,19,20,21]

Existence of a proinflammatory state was defined as a hsCRP level ≥ 1 mg/l. In addition, the hsCRP level was classified as indicative of average cardiovascular risk when it was 1-3 mg/l or as indicative of high cardiovascular risk when it was ≥ 3.0 mg/l.[22] The level of glycemic control was categorized as "reduced" when $HbA1_C$ was ≥ 5.7%; additionally, $HbA1_C$ was categorized as < 5.7% (normal), 5.7%-6.4% (prediabetes) or ≥ 6.5% (diabetes).[17] eGFR was defined decreased using the cutoff points recommended through the guidelines of the National Kidney Foundation.[23]

Continuous scores for cardiometabolic risk

The following continuous scores for cardiometabolic risk were evaluated:

- *Continuous metabolic syndrome severity Z-score (MetS Z-score)*: this was calculated by applying the equations proposed by Gurka et al.[24] for Hispanic individuals according to sex, using the calculator available at http://mets.health-outcomes-policy.ufl.edu/calculator/.
- *Cardiometabolic index (ICMet)*: Wakabayashi and Daimon[25] proposed this index. It was calculated as the product of the TGL/HDLc ratio and WHR.
- *Simple method for quantifying metabolic syndrome (siMS) score and siMS risk score)*: Soldatovic et al.[26] proposed these continuous scores. The first assesses the state of metabolic syndrome and the second evaluates the risk of coronary heart disease or cerebrovascular events. These scores were determined using the spreadsheet provided by Soldatovic et al.[26] and introducing the cutoff points of the metabolic syndrome definition applied in the present study.

Statistical analysis

Statistical Package for the Social Sciences (SPSS) software, version 20.0.0 for Windows (SPSS, Chicago, IL, USA), except for the receiver operating characteristic curves (ROC curves) and their parameters. ROC curves were obtained through the MedCalc software, in its version 13.3.3.0 for Windows.

The variables studied were assessed with regard to normality of distribution, by means of the Kolmogorov-Smirnov test. Variables that did not follow this distribution were transformed using the process described by Templeton.[27]

Means, standard deviations, medians, interquartile ranges and absolute and relative frequencies were used to characterize the sample. To correlate the frequency of cardiovascular risk factors with sex, the chi-square test was applied. The unpaired Student t test or the Mann-Whitney U test was used, as appropriate, to compare the variables according to sex, age groups and metabolic syndrome. The age groups were formed according to the median age for each sex. The Kruskal-Wallis test or Mann-Whitney U test was used, as appropriate, to compare scores according to the numbers of individual metabolic syndrome components and categories of biomarkers for cardiometabolic risk, inflammation and glomerular function.

Multiple linear regression analysis was conducted to assess the relationship between the continuous scores and the different biomarkers, with adjustment for age and sex (the back method was used for introducing variables). ROC curves for the continuous scores were constructed to test their predictive value for detecting metabolic syndrome, reduced glycemic control, proinflammatory state and decreased estimated glomerular function. The area under the curve (AUC) and its 95% confidence interval were obtained through a nonparametric method. The Hanley and McNeil method was used to compare the AUCs.

RESULTS

Table 1 shows the characteristics of the sample according to age and sex. Women showed higher age and %BF, while men had higher values for weight, height, creatinine and eGFR. Among all the subjects, 36.9% had family antecedents of cardiovascular diseases, 5.7% reported being a smoker, 29.5% were diabetic, 43.8% were hypertensive, 42.0% were undergoing hypotensive treatment at the time of the evaluation, 62.5% had metabolic syndrome, 50.6 % had reduced glycemic control, 60.6% showed a proinflammatory state and 65.3% presented decreased eGFR. Diabetes, metabolic syndrome and decreased glomerular function were more frequent among men ($P < 0.05$).

The medians for the siMS score, siMS risk score, ICMet and MetS Z-score were higher in patients with metabolic syndrome ($P < 0.001$) (**Figure 1A**). The scores studied increased as the number of individual metabolic syndrome components also increased ($P < 0.001$) (**Figure 1B**).

The variation of the scores according to the categories of the biomarkers assessed is shown in **Table 2**. All the scores were significantly higher in patients with elevated BMI, waist circumference,

Table 1. Characteristics of the study participants according to gender

Variables	Age group		Entire sample
Women	**≤ 54 years (n = 53)**	**> 54 years (n = 41)**	**(n = 94)**
Age	48.5 (44.2-52.8)**	58.0 (55.0-59.0)	54.0 (48.0-57.0)‡
Weight (kg)	67.0 (61.0-73.0)	65.0 (56.0-73.5)	66.0 (59.0-73.0)
Height (m^2)	1.56 (1.52-1.61)	1.55 (1.48-1.60)	1.56 (1.50-1.60)
BMI (kg/m^2)	27.5 (25.1-28.8)	27.3 (24.4-30.0)	27.5 (24.6-29.0)
WC (cm)	89.0 (84.5-92.0)	88.0 (81.5-94.0)	89.0 (82.8-92.2)
WHR	0.57 (0.53-0.59)	0.56 (0.52-0.61)	0.56 (0.51-0.61)
Body fat (%)	38.1 (33.4-41.0)	38.6 (36.8-44.4)	**38.5 (35.0-41.8)‡‡**
SBP (mmHg)	120.0 (102.0-139.5)	120.0 (120.0-140.0)	120.0 (110.0-140.0)
DBP (mmHg)	77.0 (70.0-83.8)	79.0 (70.0-80.0)	77.0 (70.0-80.0)
Glucose (mg/dl)	95.0 (88.2-112.5)	101.0 (89.5-113.0)	98.0 (88.8-111.8)
$HbA1_C$ (%)	5.2 (4.4-6.2)	5.9 (4.9-6.9)	5.5 (4.5-6.5)
Creatinine (mg/dl)	0.9 (0.8-1.1)	0.9 (0.8-1.0)	0.9 (0.8-1.0)
TC (mg/dl)	**215.5 (189.0-251.8)****	182.0 (152.5-234.5)	202.0 (174.5-239.5)
LDLc (mg/dl)	**129.8 (110.8-156.4)***	107.4 (82.0-148.2)	123.1 (94.2-154.2)
HDLc (mg/dl)	43.0 (37.2-54.0)	43.0 (38.0-46.5)	43.0 (38.0-50.2)
TGL (mg/dl)	187.5 (121.5-254.0)	147.0 (107.0-195.0)	163.0 (114.0-220.0)
TC/HDLc ratio	5.2 (4.1-6.1)	4.5 (3.7-5.7)	4.8 (3.7-5.9)
LDLc/HDLc ratio	3.0 (2.3-4.0)	2.7 (1.9-3.8)	3.0 (2.0-3.9)
TGL/HDLc ratio	4.3 (2.4-6.1)	3.6 (2.7-4.5)	3.8 (2.7-5.3)
Non-HDL cholesterol (mg/dl)	171.0 (148.3-195.5)**	137.0 (109.0-188.0)	158.5 (132.0-192.5)
hsCRP (mg/l)	1.4 (0.7-3.0)	1.2 (0.9-3.4)	1.2 (0.8-3.0)
eGFR (ml/min/1.73 m^2)	81.1 ± 18.4	75.6 ± 18.5	79 ± 18.6
siMS score	3.42 (3.00-3.92)	3.37 (3.01-3.63)	3.38 (3.01-3.86)
siMS risk score	3.69 (2.73-4.19)**	4.12 (3.53-4.85)	3.93 (3.24-4.54)
ICMet	2.30 (1.39-3.38)	2.08 (1.53-2.62)	2.14 (1.48-3.04)
MetS Z-score	0.61 (0.18-1.08)	0.61 (0.03-1.00)	0.61 (0.13-1.01)
Variables	**Age group**		**Entire sample**
Men	**≤ 51 years (n = 46)**	**> 51 years (n = 36)**	**(n = 82)**
Age	**47.0 (44.0-50.0)****	55.5 (53.2-58.0)	51.0 (45.8-55.0)
Weight (kg)	74.0 (69.0-84.0)	70.3 (59.0-73.0)	**72.5 (66.8-80.2)‡‡**
Height (m^2)	1.64 (1.56-1.70)	1.64 (1.55-1.70)	**1.64 (1.56-1.70)‡‡**
BMI (kg/m^2)	28.2 (25.1-30.8)	26.8 (25.0-28.6)	27.2 (25.1-30.0)
WC (cm)	94.0 (85.8-100.0)	92.0 (82.5-98.0)	92.5 (84.0-98.2)‡
WHR	0.57 (0.51-0.62)	0.56 (0.51-0.60)	0.57 (0.51-0.61)
Body fat (%)	35.3 (27.3-38.5)	34.2 (27.2-39.6)	35.0 (27.3-38.6)
SBP (mmHg)	120.0 (110.0-130.0)	120.0 (110.0-140.0)	120.0 (110.0-137.2)
DBP (mmHg)	70.0 (70.0-80.0)	70.0 (70.0-80.0)	70.0 (70.0-80.0)
Glucose (mg/dl)	109.5 (93.5-161.0)	96.0 (84.8-120.0)	101.0 (89.0-140.8)
$HbA1_C$ (%)	**6.3 (4.5-7.8)***	5.4 (4.2-6.4)	5.9 (4.3-7.3)
Creatinine (mg/dl)	1.1 (0.9-1.1)	1.0 (0.8-1.1)	**1.0 (0.9-1.1)‡‡**
TC (mg/dl)	197.0 (166.7-233.0)	196.0 (167.0-229.0)	197.0 (166.8-230.5)
LDLc (mg/dl)	122.4 (86.6-145.7)	126.4 (91.4-140.9)	125.3 (90.8-140.8)
HDLc (mg/dl)	40.0 (37.5-50.5)	45.0 (40.0-52.8)	43.0 (38.8-52.0)
TGL (mg/dl)	187.5 (130.7-224.8)	153.5 (95.8-196.5)	168.0 (116.8-204.0)
TC/HDLc ratio	4.5 (3.7-6.3)	4.1 (3.6-5.2)	4.4 (3.6-5.6)
LDLc/HDLc ratio	2.8 (2.0-4.5)	2.5 (2.1-3.5)	2.7 (2.0-3.6)
TGL/HDLc ratio	**4.4 (3.0-6.0)****	3.3 (2.0-4.3)	3.9 (2.4-5.2)
Non-HDL cholesterol (mg/dl)	161.0 (123.5-188.8)	150.5 (119.8-179.0)	152.0 (121.2-182.0)
hsCRP (mg/l)	1.7 (0.7-3.0)	1.0 (0.5-3.5)	1.2 (0.6-3.0)
eGFR (ml/min/1.73 m^2)	88.7 ± 16.2	85.7 ± 15.9	**87.4 ± 16.0‡‡**
siMS score	**3.40 (2.92-3.89)***	2.94 (2.59-3.49)	3.22 (2.71-3.66)
siMS risk score	3.64 (3.02-4.16)	3.99 (3.45-4.29)	3.75 (3.22-4.28)
ICMet	**2.35 (1.77-3.19)****	1.82 (1.11-2.34)	2.14 (1.36-2.97)
MetS Z-score	**0.66 (0.21-1.14)***	0.20 (-0.02-0.79)	0.46 (0.04-0.97)

Data expressed as mean ± standard deviation, median (interquartile range), n (%). Unpaired Student t test or Mann-Whitney U test, according to case. *P < 0.05 and **P < 0.01 between age groups. ‡P < 0.05 and ‡‡P < 0.01 between women and men.

BMI = body mass index; WC = waist circumference; WHR = waist to height ratio; SBP = systolic blood pressure; DBP = diastolic blood pressure; $HbA1_C$ = $A1_C$ hemoglobin fraction; TC = total cholesterol; LDLc = low-density lipoprotein cholesterol; HDLc = high-density lipoprotein cholesterol; TGL = triglycerides; hsCRP = ultrasensitive C-reactive protein; eGFR = estimated glomerular filtration rate.

WHR, %BF, glucose, $HbA1_C$, TGL, TGL/HDLc ratio and hsCRP; all the scores were higher among patients with low HDLc. The siMS score, siMS risk score and MetS Z-score increased significantly as eGFR decreased; these indicators were also higher in patients with proteinuria. All the scores were significantly higher among diabetic patients. Only the siMS risk score was significantly higher among smokers and patients with a family history of cardiovascular disease; none of the scores was higher in hypertensive patients.

The linear regression analysis revealed that all the scores were positively correlated with BMI, waist circumference, WHR, %BF, glucose, $HbA1_C$, TGL, TC/HDLc, LDLc/HDLc ratio, TGL/HDLc ratio, non-HDL cholesterol, hsCRP and degree of proteinuria; all the scores were negatively correlated with HDLc. The siMS score, siMS risk score and MetS Z-score were inversely correlated with the eGFR (**Table 3**). None of the scores studied showed correlations with LDLc after adjustment for age and sex.

The predictive value of the scores studied for the entire sample and according to sex are shown in **Table 4** and **Table 5**. In the entire sample, MetS Z-score, siMS score and siMS risk score showed the ability to detect or discriminate metabolic syndrome, reduced glycemic control ($HbA1_C \geq 5.7\%$), proinflammatory state (hsCRP ≥ 1 mg/l) and decreased eGFR (< 90 ml/min/1.73 m^2); ICMet only had significant capacity to discriminate patients with metabolic syndrome and a proinflammatory state. Overall, the AUCs for MetS Z-score were significantly higher than the AUCs for the rest of the scores for discriminating metabolic syndrome, decreased glycemic control and proinflammatory state. Only the AUCs for MetS Z-score and siMS score for metabolic syndrome were similar. For reduced eGFR, the AUC for the siMS risk score was greater but did not differ significantly from the AUCs corresponding to siMS score and MetS Z-score.

Among women, all the scores assessed significantly discriminated metabolic syndrome and proinflammatory state. Only MetS Z-score had the capacity to detect reduced glycemic control, while siMS risk score showed the ability to discriminate reduced eGFR. Among men, all the scores had predictive value for detecting the conditions studied.

DISCUSSION

The main purpose of this study was to examine the validity of four continuous scores that had been proposed for quantification of cardiometabolic risk. Overall, the four scores showed significant associations with most of the anthropometric and biochemical biomarkers that were measured. The scores studied showed predictive value for metabolic syndrome, reduced glycemic control, proinflammatory state and reduced estimated glomerular function, with small differences in performance especially regarding the levels of glycemic control and glomerular filtration. Metabolic syndrome was the condition for which all the scores had the greatest ability to discriminate, as expected, since all the scores were calculated using the same individual components of metabolic syndrome. In addition, all the scores increased progressively and significantly as the number of individual metabolic syndrome components increased, thus showing a continuous and gradual relationship between the scores tested and the accumulation of cardiometabolic risk factors. This behavior is desired for continuous measurements.

Out of the four scores studied, the MetS Z-score provided the most information for comparisons and for discussing its utility. In our study, after adjusting for sex and age, this score correlated with BMI, waist circumference, WHR, %BF, glucose, $HbA1_C$, HDLc, TGL, TC/HDLc ratio, LDLc/HDLc ratio, TGL/HDLc ratio, non-HDL cholesterol, hsCRP, eGFR and degree of proteinuria. In both men and women, it could detect metabolic syndrome, reduced glycemic control and proinflammatory state. These results are consistent with the associations found by Gurka et al.[24] between MetS

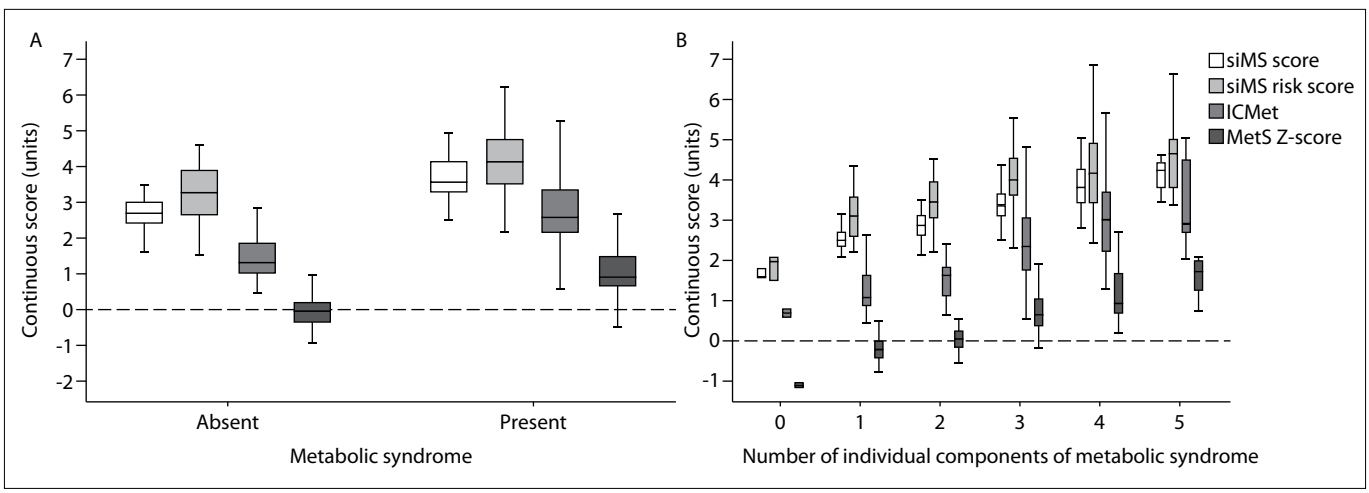

Figure 1. Continuous scores for cardiometabolic risk, evaluated according to A) presence of metabolic syndrome and B) number of metabolic components.

Z-score and risk factors, along with its ability to predict the progression of coronary heart disease and diabetes.[28,29,30,31]

The MetS Z-score can also be highlighted as having the highest AUC for detecting three of the four conditions studied. In particular, it was the only score able to discriminate $HbA1_C$ values ≥ 5.7% among women. The latter probably reflects the load factor that was obtained from glucose in constructing the equations for MetS Z-score, which was > 0.4 in women.[24]

DeBoer et al.[32] corelated elevation of the MetS Z-score with declining eGFR, higher prevalence of microalbuminuria and higher incidence of chronic kidney disease in African-American women. In our entire sample, MetS Z-score correlated negatively with eGFR

Table 2. Continuous scores for cardiometabolic risk, assessed according to biomarkers for cardiometabolic risk, inflammation and glomerular function in adult patients

Biomarkers		siMS score	siMS risk score	ICMet	MetS Z-score
BMI (kg/m²)	< 25	2.77 (2.40-3.46)	3.66 (2.66-4.12)	1.71 (1.03-2.31)	0.07 (-0.34-0.53)
	≥ 25	**3.39 (3.02-3.87)****	**3.95 (3.26-4.55)***	**2.20 (1.67-3.16)****	**0.66 (0.26-1.13)****
WC (cm)	< 90 or 80	2.99 (2.54-3.45)	3.68 (2.86-4.10)	1.81 (1.08-2.32)	0.15 (-0.24-0.57)
	≥ 90 or 80	**3.42 (3.03-3.96)****	**3.93 (3.28-4.60)***	**2.22 (1.64-3.36)****	**0.71 (0.25-1.26)****
WHR	< 0.5	2.92 (2.42-3.31)	3.58 (2.64-4.00)	1.69 (1.03-2.16)	0.05 (-0.52-0.38)
	≥ 0.5	**3.38 (2.93-3.92)****	**3.91 (3.27-4.52)****	**2.20 (1.59-3.12)****	**0.65 (0.19-1.13)****
Body fat (%)	< 25 or 30	2.48 (1.64-3.49)	3.26 (1.96-4.06)	1.08 (0.70-2.31)	-0.12 (-0.98-0.69)
	≥ 25 or 30	**3.34 (2.92-3.85)****	**3.87 (3.24-4.48)***	**2.14 (1.53-3.04)***	**0.61 (0.09-1.01)***
SBP (mmHg)	< 130	3.34 (2.71-3.86)	3.68 (3.03-4.26)	2.15 (1.36-3.24)	0.56 (0.13-1.10)
	≥ 130	3.32 (3.00-3.65)	**4.04 (3.43-4.48)***	2.12 (1.54-2.58)	0.60 (0.22-0.90)
DBP (mmHg)	< 85	3.31 (2.77-3.70)	3.86 (3.23-4.41)	2.13 (1.36-2.95)	0.53 (0.05-0.99)
	≥ 85	3.45 (3.02-3.88)	3.90 (3.12-4.48)	2.30 (1.63-3.45)	0.79 (0.26-1.04)
Glucose (mg/dl)	< 100	3.05 (2.61-3.42)	3.54 (2.80-4.11)	1.89 (1.24-2.57)	0.24 (-0.20-0.62)
	≥ 100	**3.58 (3.19-4.31)****	**4.04 (3.48-4.95)****	**2.37 (1.64-3.26)****	**0.97 (0.57-1.88)***
$HbA1_C$ (%)	< 5.7	3.18 (2.67-3.52)	3.75 (3.00-4.15)	2.01 (1.36-2.60)	0.34 (-0.10-0.65)
	5.7-6.4	3.37 (2.77-3.64)	3.69 (3.21-4.24)	1.95 (1.17-2.57)	0.64 (0.15-0.98)*
	≥ 6.5	**3.55 (3.13-4.54)**‡‡**	**4.12 (3.40-5.19)**‡**	**2.58 (1.76-3.43)*‡**	**1.03 (0.46-2.14)**‡‡**
TC (mg/dl)	< 200	3.16 (2.68-3.70)	3.67 (2.99-4.30)	1.82 (1.15-2.94)	0.48 (-0.01-0.97)
	> 200	**3.44 (3.09-3.85)***	**3.93 (3.36-4.57)***	**2.34 (1.85-3.02)****	0.61 (0.26-1.04)
LDLc (mg/dl)	≤ 130	3.31 (2.73-3.86)	3.75 (3.06-4.47)	2.08 (1.33-3.15)	0.61 (0.07-0.98)
	> 130	3.34 (2.94-3.64)	3.92 (3.30-4.34)	2.15 (1.66-2.86)	0.56 (-0.02-1.00)
HDLc (mg/dl)	> 40 or 50	2.99 (2.54-3.33)	3.49 (2.97-4.15)	1.70 (1.08-2.18)	0.22 (-0.23-0.57)
	< 40 or 50	3.56 (3.22-4.14)**	3.99 (3.43-4.72)**	2.58 (1.92-3.48)**	0.85 (0.43-1.38)**
TGL (mg/dl)	< 150	2.75 (2.44-3.15)	3.40 (2.78-3.98)	1.31 (0.99-1.79)	0.07 (-0.32-0.47)
	≥ 150	3.55 (3.30-4.21)**	4.09 (3.54-4.73)**	2.66 (2.14-3.55)**	0.79 (0.46-1.38)**
TC/HDLc ratio	≤ 5.0 or ≤ 4.5	3.06 (2.61-3.40)	3.69 (3.00-4.29)	1.72 (1.09-2.24)	0.24 (-0.11-0.72)
	> 5.0 or > 4.5	3.58 (3.33-4.14)**	3.96 (3.40-4.54)	2.62 (2.11-3.54)**	0.88 (0.46-1.34)**
LDLc/HDLc ratio	≤ 3.5 or ≤ 3.0	3.17 (2.66-3.65)	3.75 (3.06-4.29)	1.86 (1.16-2.49)	0.38 (-0.03-0.92)
	> 3.5 or > 3.0	**3.46 (3.12-4.16)****	**3.98 (3.38-4.57)**	**2.57 (1.90-3.39)****	**0.79 (0.38-1.51)****
TGL/HDLc ratio	≤ 3.0	2.67 (2.41-3.03)	3.25 (2.60-3.60)	1.17 (0.94-1.53)	-0.01 (-0.35-0.31)
	> 3.0	**3.56 (3.30-4.16)****	**4.12 (3.70-4.73)****	**2.60 (2.16-3.48)****	**0.79 (0.44-1.39)****
Non-HDL cholesterol (mg/dl)	< 130	3.13 (2.70-3.52)	3.75 (3.25-4.46)	1.72 (1.14-2.21)	0.40 (0.05-0.84)
	≥ 130	**3.41 (2.92-3.86)***	3.87 (3.18-4.43)	**2.30 (1.67-3.07)****	0.61 (0.08-1.02)
hsCRP (mg/l)	< 1.0	3.11 (2.46-3.40)	3.49 (2.83-4.09)	1.74 (1.05-2.41)	0.22 (-0.34-0.62)
	1-3	**3.46 (3.07-3.85)****	**3.77 (3.37-4.23)***	**2.29 (1.76-3.09)****	**0.66 (0.28-1.04)****
	≥ 3.0	**3.62 (3.07-4.50)****	**4.24 (3.41-5.12)**‡**	**2.52 (1.77-3.50)****	**1.02 (0.43-2.02)**‡**
eGFR (ml/min/1.73 m²)	≥ 90	3.16 (2.64-3.46)	3.32 (2.90-3.93)	1.87 (1.26-2.57)	0.31 (-0.10-0.75)
	60-89	**3.45 (2.92-4.11)****	**3.96 (3.38-4.63)****	2.24 (1.37-3.14)	**0.66 (0.16-1.44)****
	< 60	**3.38 (3.14-3.93)****	**4.08 (3.89-4.74)****	2.18 (1.68-2.77)	**0.82 (0.30-1.13)****
Proteinuria	Negative	3.11 (2.71-3.39)	3.36 (2.83-3.92)	1.85 (1.34-2.67)	0.31 (-0.01-0.70)
	1+ or more	**3.45 (2.93-3.98)****	**4.00 (3.42-4.66)****	2.19 (1.49-3.07)	**0.67 (0.09-1.36)****

Date expressed as mean ± standard deviation, median (interquartile range), n (%). Mann-Whitney U test. *P < 0.05 and **P < 0.01 with respect to the first category of the biomarker. ‡P < 0.05 and ‡‡P < 0.01 with respect to the second category of the biomarker.

BMI = body mass index; WC = waist circumference; WHR = waist to height ratio; SBP = systolic blood pressure; DBP = diastolic blood pressure; $HbA1_C$ = $A1_C$ hemoglobin fraction; TC = total cholesterol; LDLc = low-density lipoprotein cholesterol; HDLc = high-density lipoprotein cholesterol; TGL = triglycerides; hsCRP = ultrasensitive C-reactive protein; eGFR = estimated glomerular filtration rate.

and positively with the degree of proteinuria. Nevertheless, it was only able to discriminate eGFR < 90 ml/min/1.73 m^2 in males. This divergence may have been due to racial differences that affect susceptibility to deterioration of glomerular function and the distribution of the components of metabolic syndrome.

We assessed ICMet because it includes a few simple determinations that provide information on the metabolism of triglyceride-rich lipoproteins, insulin resistance and glycemic control. Wakabayashi and Daimon[25] found a positive association between ICMet and $HbA1_C$ and showed that ICMet had significant predictive value for detecting diabetes and hyperglycemia ($HbA1_C \geq 5.7\%$) in Japanese women and men. Associations between ICMet and smoking habits,[33] progression of atheromatous plaque in patients with peripheral arterial disease[34] and the risk of hypertension[35] have also been reported.

In the present study, higher ICMet was observed in individuals with $HbA1_C \geq 5.7\%$, metabolic syndrome or diabetics. Likewise, ICMet correlated with most of the biomarkers that were measured, after adjustment for sex and age (except for systolic pressure, LDLc and degree of proteinuria). However, this measurement only had the capacity to detect metabolic syndrome and proinflammatory status in the entire sample and only showed predictive value for decreased glycemic control and reduced eGFR among men. ICMet also did not vary significantly among smokers or hypertensive patients. These observations place some doubt on the applicability of ICMet as a continuous measurement

Table 4. Area under the curve for continuous scores for cardiometabolic risk of detection of metabolic syndrome, reduced glycemic control, proinflammatory state and decreased estimated glomerular filtration rate, for the entire group

Continuous scores	AUC (95% CI)	SE	P[a]	P[b]
Metabolic syndrome				
siMS score	0.930 (0.881-0.963)	0.0179	**< 0.0001**	0.2028
siMS risk score	0.763 (0.715-0.841)	0.0341	**< 0.0001**	< 0.0001
ICMet	0.871 (0.812-0.917)	0.0265	**< 0.0001**	0.0012
MetS Z-score	0.942 (0.896-0.971)*	0.0172	**< 0.0001**	----
Reduced glycemic control: $HbA1_C \geq 5.7\%$				
siMS score	0.654 (0.579-0.724)	0.041	**0.0002**	< 0.0001
siMS risk score	0.607 (0.531-0.680)	0.043	**0.0121**	0.0015
ICMet	0.574 (0.497-0.648)	0.044	0.0902	< 0.0001
MetS Z-score	0.723 (0.651-0.788)*	0.038	**< 0.0001**	----
Proinflammatory state: hsCRP ≥ 1 mg/l				
siMS score	0.779 (0.667-0.802)	0.037	**< 0.0001**	0.0036
siMS risk score	0.668 (0.593-0.737)	0.041	**< 0.0001**	0.0007
ICMet	0.677 (0.603-0.746)	0.042	**< 0.0001**	0.0002
MetS Z-score	0.780 (0.712-0.839)*	0.034	**< 0.0001**	----
Decreased glomerular function: eGFR < 90 ml/min/1.73 m^2				
siMS score	0.658 (0.581-0.779)	0.042	**0.0002**	0.6592
siMS risk score	0.676 (0.600-0.745)*	0.043	**0.0001**	----
ICMet	0.588 (0.510-0.663)	0.046	0.0562	0.0521
MetS Z-score	0.662 (0.586-0.733)	0.042	**0.0001**	0.7461

[a]Significance level for the null hypothesis AUC = 0.05; [b]significance level for comparison of AUCs with respect to the AUC that was greatest (*). AUC = area under the curve; CI = confidence interval; SE = standard error; $HbA1_C$ = $A1_C$ hemoglobin fraction; hsCRP = ultrasensitive C-reactive protein; eGFR = estimated glomerular filtration rate.

Table 3. Multiple linear regression analysis on continuous scores for cardiometabolic risk and biomarkers for cardiometabolic risk, inflammation and glomerular function, adjusted for age and sex

Biomarkers	siMS score		siMS risk score		ICMet		MetS Z-score	
	β (SE)	P	β (SE)	P	β (SE)	P	β (SE)	P
BMI	0.061 (0.014)	**< 0.001**	0.056 (0.018)	**0.002**	0.065 (0.021)	**0.002**	0.079 (0.015)	**< 0.001**
WC	0.026 (0.006)	**< 0.001**	0.022 (0.008)	**0.003**	0.026 (0.009)	**0.004**	0.037 (0.007)	**< 0.001**
WHR	4.345 (0.958)	**< 0.001**	4.236 (1.191)	**< 0.001**	4.848 (1.435)	**0.001**	5.714 (1.038)	**< 0.001**
Body fat %	0.027 (0.010)	**0.006**	0.024 (0.012)	**0.049**	0.028 (0.015)	**0.049**	0.024 (0.011)	**0.030**
SBP	---	---	0.010 (0.004)	**0.009**	---	---	---	---
DBP	0.013 (0.006)	**0.039**	0.022 (0.008)	**0.005**	0.017 (0.009)	**0.049**	---	---
Glucose	0.013 (0.001)	**< 0.001**	0.013 (0.002)	**< 0.001**	0.011 (0.002)	**< 0.001**	0.017 (0.001)	**< 0.001**
$HbA1_C$	0.176 (0.025)	**< 0.001**	0.183 (0.032)	**< 0.001**	0.158 (0.040)	**< 0.001**	0.243 (0.025)	**< 0.001**
TC	---	---	0.003 (0.001)	**0.045**	0.004 (0.002)	**0.016**	---	---
LDLc	---	---	---	---	---	---	---	---
HDLc	-0.036 (0.005)	**< 0.001**	-0.031 (0.007)	**< 0.001**	-0.052 (0.008)	**< 0.001**	-0.037 (0.006)	**< 0.001**
TGL	0.008 (0.001)	**< 0.001**	0.009 (0.001)	**< 0.001**	0.013 (0.001)	**< 0.001**	0.007 (0.001)	**< 0.001**
TC/HDLc ratio	0.243 (0.034)	**< 0.001**	0.254 (0.044)	**< 0.001**	0.392 (0.049)	**< 0.001**	0.234 (0.039)	**< 0.001**
LDLc/HDLc ratio	0.169 (0.043)	**< 0.001**	0.167 (0.054)	**0.002**	0.226 (0.064)	**0.001**	0.149 (0.048)	**0.002**
TGL/HDLc ratio	0.345 (0.017)	**< 0.001**	0.366 (0.027)	**< 0.001**	0.573 (0.012)	**< 0.001**	0.333 (0.023)	**< 0.001**
Non-HDL cholesterol	0.004 (0.001)	**0.001**	0.004 (0.001)	**< 0.001**	0.006 (0.002)	**< 0.001**	0.002 (0.001)	**0.049**
hsCRP	0.041 (0.008)	**< 0.001**	0.044 (0.010)	**0.011**	0.037 (0.012)	**0.002**	0.054 (0.008)	**< 0.001**
eGFR	-0.011 (0.003)	**0.001**	-0.013 (0.004)	**0.001**	---	---	-0.015 (0.004)	**< 0.001**
Semi-quantified proteinuria	0.724 (0.198)	**< 0.001**	0.791 (0.243)	**0.001**	0.674 (0.297)	**0.024**	0.769 (0.221)	**0.001**

SE = standard error; BMI = body mass index; WC = waist circumference; WHR = waist to height ratio; SBP = systolic blood pressure; DBP = diastolic blood pressure; $HbA1_C$ = $A1_C$ hemoglobin fraction; TC = total cholesterol; LDLc = low-density lipoprotein cholesterol; HDLc = high-density lipoprotein cholesterol; TGL = triglycerides; hsCRP = ultrasensitive C-reactive protein; eGFR = estimated glomerular filtration rate.

of cardiometabolic risk in our population. This needs to be elucidated through other studies.

The siMS score and siMS risk score were only proposed in the year 2016 and there is no further information about their performance. Their authors[26] reported that both scores correlated strongly with other indexes and that there was a medium-high grade correlation between siMS risk score and the Framingham score in a group of adult patients in Belgrade, Serbia.

In our entire sample, siMS score and siMS risk score correlated significantly with the anthropometric and biochemical biomarkers that were measured and had the capacity to detect metabolic syndrome, decreased glycemic control, proinflammatory state and reduced eGFR. However, the siMS risk score showed better performance. It was the only score that showed significant variation among smokers. In addition, the siMS risk score showed the ability to detect all the above conditions among men and to discriminate reduced glomerular filtration in women. The siMS score depends only on metabolic syndrome components. The siMS risk score is time-dependent because it incorporates age and heritability, so it is likely that its performance is better because it takes into consideration the progressive evolution of cardiometabolic risk and the genetic component involved in cardiometabolic diseases. The findings potentially support use of the siMS risk score as a continuous measurement of cardiometabolic risk, but it will be important to determine its ability to predict cardiovascular events or development of diabetes, through prospective studies.

Our attention was drawn to the fact that none of the four scores tested substantially differed with regard to LDLc and hypertension and only one (the siMS risk score) shown any significant correlation with systolic blood pressure. MetS Z-score did not have any correlation with either of the two components of blood pressure, and this finding can be partially explained by the low load factor (< 0.4) that was ascertained in relation to systolic pressure in the principal component analysis from which the equations for MetS Z-score originated.[24] In principle, these observations preclude implementation of the scores tested here, among patients with hypertension or with hypercholesterolemia alone. However, it is important to note that most of the participants in this study were undergoing treatment with antihypertensive agents or other drugs. This may have affected the results observed and therefore other investigations may be required.

The present work has some limitations. In the first place, the results found need to be confirmed through using a more extensive sample. The present results were observations derived from a group of individuals who were enrolled in a control program for chronic non-communicable diseases and their risk factors, and therefore the findings cannot be extrapolated to the general population. Secondly, the cross-sectional nature of the study and the lack of follow-up among the patients, to observe the incidence of cardiovascular events or diabetes, precluded calculation of cutoff points for stratifying the cardiometabolic risk according to the scores assessed. This latter point seems to be contradictory, given the limitations of dichotomous classifications, but it remains useful within clinical practice, for identifying patients who require strong intervention. It also forms a tangible goal for patients and their physicians, thereby functioning as a quantitative measurement of progress or deterioration.

CONCLUSION

In a sample of Venezuelan adults, all the scores studied varied according to different anthropometric and biochemical biomarkers for cardiometabolic risk. They showed predictive value for metabolic syndrome and proinflammatory status. Three scores showed a predictive capacity regarding reduced glycemic control and decreased renal glomerular function. Because this study found certain differences in the performance of the scores studied, especially with regard to sex, selection of one or another will depend on the aim and the scope pursued. The aim in follow-up studies will be to confirm the present findings and their usefulness for prevention and intervention protocols relating to cardiometabolic diseases.

Table 5. Area under the curve for continuous scores for cardiometabolic risk of detection of metabolic syndrome, reduced metabolic control, proinflammatory state and decreased estimated glomerular filtration rate, according to sex

Condition to be detected	siMS score	siMS risk score	ICMet	MetS Z-score
Women				
Metabolic syndrome	**0.926 (0.853-0.970)*****	**0.828 (0.736-0.898)*****	**0.873 (0.788-0.933)*****	**0.948 (0.882-0.983)*****
$HbA1_c \geq 5.7\%$	0.577 (0.471-0.679)	0.579 (0.473-0.680)	0.515 (0.409-0.619)	**0.649 (0.544-0.745)****
hsCRP ≥ 1 mg/l	**0.706 (0.603-0.796)****	**0.687 (0.52-0.779)****	**0.642 (0.536-0.779)***	**0.775 (0.677-0.855)****
eGFR < 90 ml/min/1.73 m^2	0.597 (0.489-0.699)	**0.680 (0.489-0.699)****	0.525 (0.417-0.631)	0.594 (0.486-0.696)
Men				
Metabolic syndrome	**0.935 (0.859-0.978)*****	**0.733 (0.624-0.825) *****	**0.884 (0.794-0.944)*****	**0.947 (0.874-0.984)*****
$HbA1_c \geq 5.7\%$	**0.753 (0.646-0.842)*****	**0.655 (0.542-0.757)****	**0.648 (0.535-0.751)****	**0.814 (0.712-0.891)*****
hsCRP ≥ 1 mg/l	**0.769 (0.663-0.855)*****	**0.642 (0.528-0.745)***	**0.721 (0.611-0.815)*****	**0.789 (0.685-0.872)*****
eGFR < 90 ml/min/1.73 m^2	**0.713 (0.600-0.809)*****	**0.667 (0.552-0.769)****	**0.656 (0.540-0.759)****	**0.732 (0.620-0.825)*****

Data expressed as AUC (95% confidence interval).

*P < 0.05 for the null hypothesis AUC = 0.05. **P < 0.01 for the null hypothesis AUC = 0.05. ***P < 0.0001 for the null hypothesis AUC = 0.05.

AUC = area under the curve; $HbA1_c$ = $A1_c$ hemoglobin fraction; hsCRP = ultrasensitive C-reactive protein; eGFR = estimated glomerular filtration rate.

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Prevalence study on self-declared work accidents in areas covered by family health strategies: a cross-sectional study

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ABSTRACT

BACKGROUND: Occupational accidents are a complex phenomenon and a major public health problem. Occupational health surveillance actions are essential for prevention of injuries of this nature.

OBJECTIVE: To ascertain the prevalence of and the variables associated with occupational accidents in the city of Itajubá (MG).

DESIGN AND SETTING: A cross-sectional study with a quantitative approach, based on a household survey with random sampling, was conducted in areas covered by the Family Health Strategy (FHS) in Itajubá (MG).

METHODS: Questionnaires were applied to 292 people. The data were analyzed by means of logistic regression.

RESULTS: The prevalence of occupational accidents was 8.6%. The underreporting rate was 60.0%. The scenario for these accidents, according to the model established through the regression analysis, was most likely to involve males who declared their skin color as white and who did not have a formal employment contract.

CONCLUSION: This study makes a contribution towards unveiling the relationship between healthcare and work, and thus serve as support for the development of strategies to prevent underreporting. Lastly, the results provide the basis for future public health intervention actions and for future studies.

INTRODUCTION

Occupational accidents are a complex phenomenon and a major public health problem that requires attention from all sectors, including healthcare, industry or services, because these accidents generate high socioeconomic costs.[1]

Work that is carried out under unsuitable conditions can cause various adverse consequences for humans, both physically and psychologically. These consequences may give rise to impairments that lead to absenteeism, premature retirement, reduced income, job disruption for the family, temporary incapacitation, physical or psychological pain, mutilation and even death. Faced with this reality, it is essential that this topic is discussed in order to conduct studies that show the unfavorable impacts of work on workers' health.[2,3] One of the main causes of health problems among workers is the fragility of the health and safety structure, along with inadequate risk management.[4]

In order to implement care, surveillance, preventive actions and health promotion actions, the National Network for Integral Attention to Worker's Health (RENAST)[5] was created in Brazil in 2002. This is a complex network that includes production and management of knowledge and actions to be developed for workers' health. The network is composed of state and regional reference centers for occupational health (CERESTs) that have the objective of acting towards health promotion, preventive actions, surveillance, assistance and rehabilitation relating to workers' health in both urban and rural settings, irrespective of employment relationships and the type of status in the labor market.[6]

All work accidents must be reported in the notifiable hazards information system (Sistema de Informação de Agravos de Notificação, SINAN). This system has been implemented gradually since 1993 and is mainly fed by notifications and investigations of cases of diseases and injuries that are listed as matters for compulsory notification. When used effectively, this system makes it possible to identify the epidemiological reality of a given geographical area.[7]

In the case of accidents among workers who are insured through social security, it is necessary to open a work accident notice (Comunicação de Acidente de Trabalho, CAT). The aim

of this is to inform the social security system about the occurrence of an accident at work, even if this event does not lead to a worker's absence.[8]

Because health problems among workers can give rise to a variety of types of harm and consequences, it is necessary to identify the associated factors. The official statistics on occupational accidents are insufficient to delineate such problems in an in-depth and accurate manner that allows the realities to be understood. It is important to note that underreporting occurs, especially when it comes to workers who are not covered by social security.[9] Most of the time, the reliability of information among government agencies is compromised because of the large numbers of informal workers with no employment relationship.[3]

Occupational health surveillance actions are essential for prevention of injuries of this nature. Persistent underreporting of occupational accidents, which is a public health problem, constitutes a significant obstacle to the effectiveness of these measures. It also has a harmful and important repercussion on labor and social security rights.[10]

OBJECTIVE

To ascertain the prevalence of and the variables associated with occupational accidents in the city of Itajubá (MG), Brazil.

METHODS

Place of study

The study was conducted in the municipality of Itajubá, which has a population of 90,658 inhabitants and is located in the southern region of Minas Gerais.[11] The industrial district of Itajubá is considered one of the largest in the south of Minas Gerais, with large and medium-sized industries, in the fields of auto parts, military equipment, helicopters and soaps, among others, generating approximately 2,500 jobs.

Study population

The study population comprised the population that is covered by the Family Health Strategy (FHS), which covers approximately 41.33% of the inhabitants of the municipality, including both the rural and the urban area, with 13 FHS units located in the urban area and two FHS units in the rural area.[12]

Study design

This was a cross-sectional quantitative study based on a household survey.

Sample definition and data collection

The sample size was determined based on the method proposed by Gil.[13] Through this method, it was defined as 265 subjects. A safety margin of 10% was also taken into consideration in relation to the sample size, thus resulting in a total of 292 homes to be visited.

The data collection was based on the method used by Cordeiro et al.,[10] in which the steps were the following:

- Preliminary survey of FHS data to identify whether homes were occupied or unoccupied;
- Sample definition by means of a simple random process;
- Data collection.

Through the data collection, which was performed through application of individual questionnaires in September 2015, it was sought to reveal the prevalence and underreporting of accidents. Closed homes, i.e. those that were classified as occupied, but with no residents present at the time of the visit, were visited again on up to two more occasions, on different days and at different times. If, on the third visit, the home was still closed, it was discarded from the sample and replaced. Among the homes visited, if the resident (aged eighteen years or over) refused to participate, this home was discarded from the sample and replaced.

Subjects who agreed to respond to the questionnaire were asked whether any of the residents of their household over the age of sixteen years had suffered any accident of any kind (work, traffic, domestic, etc.) in the last two years. If so, a return to the address was scheduled, to interview this individual and confirm the occurrence of the alleged accident. When its occurrence was confirmed, a semi-structured questionnaire was applied to the subject involved in the event.

A pre-test was performed using 20 subjects in different homes in order to ascertain the adequacy of the questionnaire and to validate. The questionnaire had been drawn up by the authors of the present study. This process was undertaken to evaluate the clarity of the questions and avoid redundancy among them, and to assess the effectiveness of the questionnaire.

The questionnaires were applied by the authors themselves, with the support of a duly trained research auxiliary. The aim was to identify work accidents that occurred during the period from August 2013 to August 2015.

The criteria for subjects to be included in the study were that they needed to have lived in Itajubá for at least one year and to have had occupational activity in Itajubá for at least one year; and that their homes were covered by the FHS. The criteria for them to be excluded were that they had lived in Itajubá for less than one year or carried out occupational activities in another municipality; and that their homes were not covered by the FHS.

For the respondents who were paying social security contributions and had suffered an occupational accident, the proportion of underreporting was calculated as the ratio between the observed number of non-notified work accidents that should have been

subject to compulsory notification and the total number of events (reported and not reported).

Data analysis

The associations between the dependent variable (work accidents suffered during the two-year period) and the explanatory variables were studied through multiple non-conditional logistic regression, to make it possible to control for confounding variables, in order to avoid possible interferences regarding the outcome of the study.

The variables for which there were more than two response options were dichotomized at the time of their insertion in the EPI-INFO software, version 3.5.1TM (2008). Thus, marital status was separated into married/stable partnership versus single/separated; skin color into black versus white/brown; and employment status into registered versus unregistered/self-employed.

Ethical issues

The present study followed all the ethical principles, and data collection was only done after approval had been obtained from the Research Ethics Committee of the Medical School of Itajubá (MG), under the constituted opinion number 1,111,978 (approval date: June 17, 2015). Resolution 196/96 and the principles of autonomy and privacy were respected. People who agreed to participate in the study signed an informed consent statement.

RESULTS

Among the questionnaires applied, 292 were considered valid. The sociodemographic data of these 292 study participants, whose mean age was 45.1 ± 16.8 years, and the prevalence of underreporting are shown in **Table 1**.

There were 25 workers who had suffered industrial accidents, and the distribution of their characteristics is shown in **Table 2**. The predominant characteristic of these individuals who sustained injuries during the observation period was that they were married white men who had not been trained to perform the function.

Among all the 25 workers who suffered an accident at work, 12 were not registered or were self-employed, and five of these were paying social security contributions but did not notify SINAN. The other 13 workers had formal employment contracts and 10 did not open a CAT after their accident. Thus, in total, 15 workers among these 25 accident victims did not open a CAT or make the mandatory notification to SINAN, thereby representing an underreporting rate of 60.0%.

Logistic regression analysis

The model that was considered to present the most appropriate fit was one that considered the accidents that occurred over the entire period of the study.

Table 3 shows the results obtained by means of the univariate and multiple analysis, which shows the respective prevalence odds ratio, 95% confidence interval (CI) and P-value of the explanatory variables.

DISCUSSION

The prevalence of occupational accidents over the two-year period from August 2013 to August 2015 was 8.6%: 3.5% in the first year and 5.1% in the second year. The annual values found are close to those reported from the study developed by Binder et al..[14] On the other hand, Cordeiro et al.[10] found that the prevalence of typical work accidents over a three-month period was 0.7%. It is important to mention that these two studies, which included the whole municipality, used different methodologies regarding the definition of sampling. Differently, the present study had the particular feature of only involving people who were covered by the FHS.

Among the 25 workers who suffered work accidents, 15 workers (10 with employment registration and five without registration

Table 1. Distribution of the sociodemographic data of the 292 interviewees and of the prevalence of work accidents according to stratum, in Itajubá (MG) between August 2013 and August 2015

Explanatory variable	n (%)	Prevalence of work accidents by stratum n (%)
Skin color		
White	168 (57.7)	20 (11.9)
Brown	114 (39.0)	4 (3.5)
Black	10 (3.4)	1 (10.0)
Marital status		
Single	88 (30.1)	5 (5.7)
Married	178 (61.0)	16 (9.0)
Separated	7 (2.3)	1 (14.3)
Stable partnership	3 (1.0)	3 (100.0)
Widower	16 (5.5)	0 (0.0)
Schooling		
Elementary	135 (46.2)	12 (8.9)
High School	144 (49.4)	13 (9.0)
Higher education	6 (2.1)	0 (0.0)
Illiterate	7 (2.3)	0 (0.0)
Location of home		
Urban area	256 (87.8)	24 (9.4)
Employment relationship		
Working with formal employment contract	104 (35.6)	13 (12.5)
No employment contract	52 (17.9)	8 (15.4)
Self employed	21 (7.3)	4 (19.0)
Retired	50 (17.1)	0 (0.0)
Unemployed	65 (22.1)	0 (0.0)
Gender		
Male	118 (40.5)	20 (6.8)
Underreporting of work accidents	60.0%	
Total	**292 (100)**	**25 (8.6)**

or self-employed) did not open a CAT or give notification through SINAN. This figure is equivalent to a prevalence of underreporting of 60.0%, which is a very worrying proportion, since it shows that more than half of the work-related accidents were not reported. Of this proportion, 55.6% belong to the category of workers with registration and 27.7% to workers without registration or who were self-employed who were nonetheless paying contributions to the National Social Security Institute. Among the five workers who were making social security contributions but who were unregistered or self-employed, none of them made a notification of the accident.

Table 2. Distribution of the sociodemographic characteristics of the 25 workers who suffered an accident at work, Itajubá (MG), 2013-2015

Explanatory variable	n (%)
Not trained	18 (72.0)
Medical care	
Hospital	17 (68.0)
Outpatient	1 (4.0)
In the company	6 (24.0)
Primary healthcare unit	1 (4.0)
Length of time in the profession in years [mean (standard deviation)]	8.9 (8.7)
Hours elapsed from the beginning of work to the time of the accident [mean (standard deviation)]	4.9 (2.9)
With remission from work	15 (60.0)
Days of absence [mean (standard deviation)]	42.9 (96.2)
Place of work accident	
Industry (factories and machine shops)	8 (32.0)
Construction	8 (32.0)
Butcher's shop	3 (12.0)
Restaurant	2 (8.0)
Family home	1 (4.0)
Laboratory	1 (4.0)
School	1 (4.0)

Table 3. Univariate and multiple analyses presenting odds ratios, 95% confidence intervals and P-values for the dependent variable "work accidents over the two-year period", Itajubá (MG), 2013-2015

Univariate analysis				
Explanatory variables	**OR***	**95% CI**	**P-value**	
Skin color (white)	3,13	1.15-8.52	0.015	
Marital status (married)	1.14	0.49-2.66	0.75	
Schooling (high school)	0.97	0.43-2.19	0.95	
Location of home (urban area)	3.51	0.46-26.54	0.14	
Formal employment contract	0.51	0.33-0.77	< 0.001	
Gender (male)	6.59	2.42-17.96	< 0.001	
Age	0.97	0.95-10.02	0.06	
Multiple analysis				
Explanatory variables	**OR****	**95% CI**	**Coefficient (β)**	**P-value**
Gender (male)[1]	6.28	2.25-17.50	1.84	0.0005
Formal employment contract[1]	0.05	0.03-0.08	- 0.07	0.0024
Skin color (white)[1]	3.63	1.28-10.30	1.29	0.0152

OR = odds ratio; CI = confidence interval.
[1]The variables that were entered in the adjustment; *Crude odds ratio; **Adjusted odds ratio.

Underreporting compromises public policies for promoting workers' health. Among the main reasons for underreporting are an understanding that the event was not of sufficient severity,[15] a duration of sick leave of less than sixteen consecutive days, for which there is no concession of benefits,[16] occurrence of accidents among informal workers,[17] poor functioning of health surveillance for workers and weak capacity and responsibility among municipalities for adequate identification and notification of cases.[18]

The National Health System (Sistema Único de Saúde, SUS), through the FHS, has a very important role in identifying underreporting of occupational accidents, since primary care is the gateway for people to access healthcare services. Hence, it needs to be possible for the entire community covered to be received within primary care, so that links between patients and professionals can be established. Community health agents work side-by-side with the community and so are able to be better informed about whether occupational accidents have occurred or not. In addition, a relationship of trust is established through coexistence between these health agents and patients. Consequently, health agents can obtain a greater amount of information regarding what is happening with regard to the health of members of the community who live in the homes that are covered.

Another way to improve surveillance relating to occurrences of occupational accidents is to implement additional CERESTs. These centers are specialized places that have a responsibility for hosting these workers, thereby clarifying their doubts and acting towards promotion, protection and recovery of workers' health.

Regarding the prevalence of disability due to work accidents, a proportion of 60% was found, similar to what was found in another study.[19]

As observed in the present study, Rios et al.[15] also found a greater number of work accidents among men. However, another study[20] found that these accidents were predominantly among women. This finding can be explained by the fact that the latter study was carried out among healthcare professionals, among whom women predominate.

The average age of the victims was 38.7 years (standard deviation, SD = 10.05), as has also been found in other studies,[15,20-22] in which mean ages of between 33 and 39 years were found.

Regarding skin color, white skin color prevailed in the present study. This result differed from what was found by Santana et al.,[23] who observed higher prevalence of accidents involving black-skinned people in Salvador, Bahia. The explanation for this difference is that the city of Itajubá has a predominantly white population, while the region of Salvador has a population of black and brown skin colors.[24]

Regarding schooling, Santana et al.[23] observed that the prevalence was 72% among subjects with less than high school education.

This differed from what was found in the present study, in which the proportion was 48%.

Regarding medical care, most of the patients were attended to in the emergency room, unlike in another study,[23] in which the majority received care in outpatient clinics. This may indicate the possibility that the work accidents in Itajubá were more serious.

Most of the accident victims were treated within the public healthcare network, as was also found in another study.[10] The public healthcare network can act as an essential support for strengthening the information system on occupational accidents.[10] In addition, in line with these measures, CERESTs that are properly organized and structured have an important role in strategies contributing towards notification of occupational accidents in all municipalities.[25] Demonstrating The importance of implementing a CEREST in the place of the present study was thus demonstrated, with the aim of reducing the level of underreporting.

In the current survey, 32% of the accidents occurred in environments and processes relating to industrial activity, and another 32% in construction. In a study by Binder et al.,[14] the secondary sector (i.e. industrial activity) accounted for 42.1% of accidents, while construction accounted for 18.4%. In the present study, working as a butcher took third place regarding the prevalence of accidents. Rios et al.[15] found a strong association between occurrences of accidents and working as a butcher, which can be explained in terms of the risks present in the work process and poor management of these risks.

The main concern regarding occupational accidents is that many of the workers who suffer these accidents do not have any formal employment contract (46%). Thus, they are sometimes left homeless and without income when these accidents occur.[26,27]

Regarding the variable of age, although this was an important biological factor, it was decided not to place it in the multiple analysis, so that the significance of the model would not be reduced.

Through logistic regression, it was possible to observe that male workers were more likely to suffer work accidents, as had already been observed in the literature.[21-23,28-32]

Regarding the situation of being registered or not, it was observed that registration acted as a protection factor in relation to work accidents. No association was found between these two factors (registration and work accidents) in the literature. Therefore, this possible association deserves to be better addressed in future research, with the hypothesis that there is a relationship between work accidents and informal work. The idea would be that when worker are registered, this presupposes there is greater concern on the part of the employer about adoption of preventive measures. It should also be noted that outsourced workers are registered. Outsourcing is another important variable that has not been addressed, and this should be considered in future research on the genesis of occurrences of accidents.

White skin color was also a significant factor in increasing the likelihood of occurrences of accidents, as had previously been observed in other studies.[33,34]

Lastly, as limitations of the present study, it can be noted that it had the typical limitations of a cross-sectional study, such as information bias and memory bias, since information about workers who have suffered accidents at work needs to be validated through medical records or diagnoses. The present study depended exclusively on the memories of the people who were interviewed.

CONCLUSION

Our study found that the prevalence of occupational accidents in the city of Itajubá (MG) was close to what had been seen in the literature. In relation to the variables associated with occupational accidents, the most favorable scenario for occurrences of a work-related accident, found through logistic regression, consisted of situations in which work was done without a formal contract, by males and by people with white skin color. Having a formal employment contract acted as a protection factor.

It is recommended that further studies should be conducted, with exploration of other variables, such as outsourcing, the relationship between work accidents and lack of a signed employment contract, among others that may help in explaining the work accident phenomenon.

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Use of smartphone-based instant messaging services in medical practice: a cross-sectional study

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ABSTRACT

BACKGROUND: Instant messaging services (IMS) are widely used in medical practice.

OBJECTIVE: To evaluate perceptions regarding use and usability of IMS within clinical practice and assess users' knowledge of the ethical and legal context involved in using IMS within medical practice.

DESIGN AND SETTING: Cross-sectional study conducted in different hospitals and medical institutions in Minas Gerais, Brazil.

METHODS: Medical students, medical residents, primary care physicians and specialist doctors answered an online questionnaire regarding epidemiological data, graduation level and use of IMS for medical communication. Responses were collected over a five-month period and data were assessed using the IBM-SPSS software.

RESULTS: 484 people answered the questionnaire: 97.0% declared that they were using IMS for medical-related purposes; 42.0%, to elucidate medical concerns every week; 75.0%, to share imaging or laboratory tests and patients' medical records; and 90.5%, to participate in clinical case-study private groups. Moreover, only 37.0% declared that they had knowledge of the legislative aspects of use of smartphones within clinical practice. Differences in the frequency of discussion of medical concerns within the daily routine between student/residents and general practitioners/specialists, and in the frequency of image-sharing and patient-guiding/assistance between students and medical doctors, were observed.

CONCLUSIONS: Our results provide reliable proof that medical doctors and students use IMS, as a tool for clinical case discussions, interactions between healthcare providers and patients, or dissemination of knowledge and information. Nonetheless, because of limitations to the ethical and legal regulations, evidence-based discussions between authorities, academics and medical institutions are needed in order to fully achieve positive outcomes from such platforms.

INTRODUCTION

Use of telemedicine has become more frequent and more convenient for addressing medical issues.[1] It is perfectly suited for use in the modern world because of its cost-efficiency, its availability in remote and rural areas, the improved access to care that it provides and its shorter response time. It can also improve the consistency and quality of healthcare.[2]

In 2018, data from the International Telecommunications Union showed that there were more than 3.9 billion active mobile phone (cellphone) subscriptions worldwide.[3] More than 165,000 health-related applications (apps) have been designed and 62% of smartphone holders use their phone to obtain health advice.[4,5]

Social media and smartphone-based instant messaging services (IMS) have exploded in popularity over recent years. Instant messaging services, such as WhatsApp and iMessage, have become a very common way to communicate, for personal and professional purposes.[6] The use of these services has become progressively more popular within the field of medicine, and they serve to connect doctors to patients, to other doctors and to other healthcare professionals.[7] This paradigm shift in medicine, created through popular communication applications, is of relevance both to developed and developing countries because of the economic, political and social issues that arise through use of these means of communication.

Within the context of telemedicine programs, it is important to consider the legal perspective relating to contact between healthcare professionals and their patients or between these professionals and their colleagues. There is a need to avoid problems relating to privacy issues and medical malpractice, as well as to avoid fraud and abuse.

In Brazil, the Federal Medical Council (Conselho Federal de Medicina, CFM) recently raised concerns regarding indiscriminate use of these apps among healthcare professionals and their patients.[8] In April 2017, the CFM published a memorandum (reference number 14/2017) regulating the use of IMS for physician-physician communication and for patient care. This policy states that these apps must not replace face-to-face consultations: they should be a complement to regular medical practice. More recently, the promulgation of a data protection law in Brazil, to regulate the use of personal information by third parties, has also given rise to more discussion on the use of data for medical purposes.[9] It is notable that sensitive personal information is commonly exposed, whether in social media, on television channels or between companies.[10,11] This is symptomatic of the current lack of legal information and boundaries for medical doctors with regard to information sharing. No research so far has analyzed such unawareness among health professionals.

OBJECTIVE

To ascertain perceptions regarding use and usability of these apps within clinical practice and to assess users' levels of knowledge about the ethical and legal context involved in use of these apps within medical practice.

METHODS

This was a cross-sectional study conducted in the state of Minas Gerais, in southeastern Brazil. The inclusion criteria were that the subjects needed to be any of the following: (1) medical students at a public university in Belo Horizonte, the state's capital and largest city, with 2.5 million inhabitants; (2) medical residents at this university's teaching hospital; (3) primary care physicians registered in the database of the Telehealth Network of Minas Gerais (TNMG), which is a public Telehealth service that was providing primary care services for 814 municipalities in Minas Gerais at that time; or (4) specialist doctors at the university's teaching hospital or registered in the database of the TNMG.

A standardized questionnaire containing 10 closed-ended questions and 4 open-ended questions was developed by an interdisciplinary group of specialists. It was then hosted in a survey administration application (Google Forms). In total, 6591 e-mails were sent out containing an invitation to participate, a description of the research and an access link to the questionnaire.

The questions addressed the following: age; professional experience; frequency of instant messaging service use for medical-related purposes (in days); participation in health-related app groups (number of groups involved); use of instant messaging apps for clinical-support tools (yes/no), or for patient follow-up care or monitoring; and perception of benefits from these apps for enabling clinical solutions (using a defined scale). In addition, one question investigated the subjects' level of knowledge of the legal aspects of sharing and discussing medical matters using instant messaging apps. An invitation to participate in the survey was sent out electronically to eligible respondents, who had been identified through the academic office of the medical school, the residency program office of the university hospital and the TNMG.

All the data were assessed using the IBM Statistical Package for the Social Sciences (SPSS) for Windows, version 19.0. Categorical variables were presented as absolute numbers and relative frequencies and continuous variables as medians and interquartile ranges, since the distribution was not normal. The participants were categorized according to their level of education as medical students, medical residents, medical specialists and general physicians. The Kolmogorov-Smirnov test and Kruskal-Wallis test were used to assess differences among groups. We decided to compare two main clusters of participants: medical students and medical residents versus specialists and general practitioners. This was because of the possible correlations and similarities of patterns among these individuals (whether still graduating or professionally restricted).

This investigation was approved by the local Research Ethics Committee, through protocol number 82097018.0.000.5149, and consent was obtained from all participants in accordance with the Helsinki Declaration.

RESULTS

The total study population consisted of 484 people. The median age was 27 years (interquartile range, IQR 23-33), and most of the participants were 20-30 years of age (60.0%). With regard to the respondents' educational level, 41.0% were medical students, 22.0% resident physicians, 21.0% specialist doctors and 16.0% general doctors. Overall, among the medical students (n = 197), 36.0% were in the fifth or sixth year of medical school, 32.5% in the third or fourth year and 31.5% in the first or second year. Among the physicians, a considerable proportion of the participants stated that they had between 0 and 5 years of experience (33.0%).

Table 1 shows details of the subjects' responses relating to usage of instant messaging services, according to educational status. Most respondents (97.0%) declared that they were using these apps for medical-related purposes, with higher prevalence among residents/students than among general practitioners/specialist s(298 versus 163; $P \leq 0.001$). There was a significant difference between medical students/residents and general practitioners/specialist regarding frequency of use for medical purposes, except for irregular daily use. The participants' frequency of use of instant messaging apps for medical purposes was categorized as a few times a day or multiple daily access. Additionally, 42.0% of the participants reported that they were using instant messaging services every week to elucidate medical concerns, in the form of

Table 1. Online questionnaire responses according to multiple categories

	Total (n = 474)	Medical student (n = 197)	Medical resident (n = 103)	Medical specialist (n = 98)	General practitioner (n = 76)	Student/resident versus general physician/ specialist (P-value)
Use of instant medical communication app	461 (97.2)	195 (99.0)	103 (100.0)	90 (91.8)	73 (96.1)	≤ 0.001
Use of WhatsApp	460 (97.0)	195 (99.0)	103 (100.0)	90 (91.8)	72 (94.7)	≤ 0.001
Use of Facebook Messenger	272 (57.3)	132 (67.0)	58 (56.3)	51 (52.0)	31 (40.8)	≤ 0.001
Use of Skype	53 (11.1)	22 (11.0)	8 (1.0)	15 (15.0)	8 (10.0)	0.283
Use of Telegram	66 (13.9)	25 (12.0)	16 (15.0)	15 (15.0)	10 (13.0)	0.831
Use of iMessage	39 (8.2)	15 (7.0)	9 (9.0)	9 (9.0)	6 (8.0)	0.812
Use of Viber	15 (3.1)	1 (0.01)	8 (8.0)	5 (5.0)	1 (1.0)	0.788
Use of Hangouts	14 (2.9)	9 (4.0)	1 (1.0)	4 (4.0)	0 (0.0)	0.521
Frequency of use for medical purposes						
No use	15 (3.2)	2 (1.0)	0 (0.0)	10 (10.2)	3 (3.9)	≤ 0.001
Rare	44 (9.2)	8 (4.1)	3 (2.9)	17 (17.3)	16 (21.1)	≤ 0.001
A few times a day	177 (37.4)	78 (39.6)	43 (41.7)	25 (25.5)	31 (40.8)	0.077
Multiple daily access	238 (50.2)	109 (55.3)	57 (55.3)	46 (46.9)	26 (34.2)	0.003
Number of discussion groups involved						
No group	45 (9.3)	13 (6.6)	1 (1.0)	22 (22.4)	9 (11.8)	≤ 0.001
1-2 groups	100 (20.7)	25 (12.7)	11 (10.7)	36 (36.7)	28 (36.8)	≤ 0.001
3-5 groups	182 (37.6)	87 (44.2)	46 (44.7)	19 (19.4)	30 (39.5)	≤ 0.001
More than 5 groups	147 (30.4)	72 (36.5)	45 (43.7)	21 (21.4)	9 (11.8)	≤ 0.001
Frequency of online discussion of clinical cases						
No discussion	42 (8.7)	20 (10.2)	1 (1.0)	16 (16.3)	5 (6.6)	0.061
Rarely	80 (16.5)	28 (14.2)	12 (11.7)	23 (23.5)	17 (22.4)	0.007
Daily	90 (18.6)	31 (15.7)	28 (27.2)	23 (23.5)	8 (10.5)	0.620
Weekly	199 (41.1)	87 (44.2)	52 (50.5)	22 (22.4)	38 (50.0)	0.012
Monthly	63 (13.0)	31 (15.7)	10 (9.7)	14 (14.3)	8 (10.5)	0.752
Perception of use						
Never used	43 (8.9)	20 (10.2)	2 (1.9)	16 (16.3)	5 (6.6)	0.653
Never helped	7 (1.4)	3 (1.5)	2 (1.9)	1 (1.0)	1 (1.3)	0.084
Used but could solve the case without the application	157 (32.4)	77 (39.1)	29 (28.2)	30 (30.6)	21 (27.6)	0.179
Used and considered essential	267 (55.2)	97 (49.2)	70 (68.0)	51 (52.0)	49 (64.5)	0.703
Use for image-sharing purposes						
No use	115 (23.8)	71 (36.0)	6 (5.8)	22 (22.4)	16 (21.1)	0.349
Rarely	122 (25.2)	50 (25.4)	26 (25.2)	24 (24.5)	22 (28.9)	0.791
Daily	44 (9.1)	12 (6.1)	12 (11.7)	16 (16.3)	4 (5.3)	0.206
Weekly	136 (28.1)	45 (22.8)	45 (43.7)	25 (25.5)	21 (27.6)	0.408
Monthly	57 (11.8)	19 (9.6)	14 (13.6)	11 (11.2)	13 (17.1)	0.367
Frequency of patient-guiding orientation						
No use	234 (51.0)	145 (74.0)	50 (49.0)	27 (27.6)	23 (30.3)	≤ 0.001
Rarely	134 (27.7)	37 (19.0)	35 (34.0)	32 (32.7)	30 (39.5)	0.007
Daily	22 (10.7)	3 (2.0)	1 (1.0)	14 (14.3)	4 (5.3)	≤ 0.001
Weekly	44 (9.2)	5 (2.5)	8 (8.0)	21 (21.4)	10 (13.2)	≤ 0.001
Monthly	26 (5.4)	5 (2.5)	8 (8.0)	4 (4.1)	9 (11.8)	0.148
Legal knowledge						
No legal knowledge	121 (25.5)	59 (29.9)	25 (24.3)	18 (18.4)	19 (25.0)	0.105
No literature-based knowledge	180 (38.0)	82 (41.6)	47 (45.6)	23 (23.5)	28 (36.8)	0.003
Literature-based knowledge	173 (36.5)	56 (28.4)	31 (30.1)	57 (58.2)	29 (38.2)	≤ 0.001
Impact on medical practice						
No impact	17 (3.6)	2 (1.0)	0 (0.0)	11 (11.2)	4 (5.3)	≤ 0.001
Negative impact	6 (1.2)	2 (1.0)	1 (1.0)	2 (2.0)	1 (1.3)	0.497
Either positive or negative	342 (72.2)	162 (82.2)	85 (82.5)	51 (52.0)	44 (57.9)	≤ 0.001
Positive impact	109 (23.0)	31 (15.7)	17 (16.5)	34 (34.7)	27 (35.5)	≤ 0.001

Values shown are n (%) or median (interquartile range).

either professional-to-professional interactions or group settings, with higher weekly prevalence among medical student/residents than among specialists/general practitioners (41.9% versus 12.6%; P = 0.012 respectively). Most of the respondents perceived advantages from using these apps in clinical practice (55.2%), while 1.4% of those who had already used instant messaging apps considered that they did not help whatsoever within clinical practice (i.e. they believed that these apps did not provide any reasonable benefits).

Regarding perceptions of use, there were no statistically significant differences between medical students/residents and specialists/general practitioners. Most of the respondents also declared that they had already used these apps to sharing imaging examinations, laboratory tests or patients' medical records (76.2%), with no statistically significant differences between medical students/residents and general practitioners/specialists. Additionally, most of the participants belonged to clinical case-study closed groups (90.5%), and frequently more than three groups. Interestingly, medical students/residents were more likely to participate in more than three discussion groups, while general practitioners/specialists more frequently belonged to one or two discussion groups.

A total of 50.6% of the participants stated that they had previously used smartphone-based instant services for guiding and/or advising patients. Lower daily and weekly use of instant messaging apps for patient-guiding was seen among medical students/residents than among general practitioners/specialists (25.75% versus 74.25%; $P \leq 0.001$).

With regard to opinions about the impact of instant messaging apps on clinical practice, 72.2% of the respondents considered that these apps could have either a positive or a negative impact on medical practice, while 22.5% considered that these apps were entirely positive. Medical students/residents were less likely to perceive the positive impact of instant messaging apps than were general practitioners/specialists (44.0 versus 55.9%; $P \leq 0.001$).

With regard to knowledge of ethics and legal matters, 74.5% of the participants stated that they had previous knowledge of the legislative aspects of use of these apps, although 38.0% of these participants reported that they had never checked this in the official literature. The remainder of the participants stated that they did not have any previous information relating to legal perspectives (25.5%). Regarding legal knowledge gained from the literature, there was a statistically significant difference between medical students/residents and specialists/general practitioners (18.3% versus 18.1%; $P \leq 0.001$).

DISCUSSION

Studies on the impact of instant messaging apps within medicine are rare and still at an initial stage. In the present study, most of the physicians and medical students reported that they were using instant communication apps for medical-related issues, and WhatsApp and Facebook Messenger were the ones most used. The majority of the respondents reported that they were using instant messaging technologies to participate in discussion groups, for a variety of purposes: imaging sharing (radiological, clinical or laboratory), clinical case discussion, knowledge dissemination and, more rarely or not at all, for patient-guiding. Use of instant messaging apps was mostly perceived and evaluated as useful/essential or useful but not mandatory and the perception of negative impacts on medical practice was remarkably low. Most of the participants had not seen any literature-based evidence regarding the use of these apps.

The fact that most of the participants declared that they were using WhatsApp and Facebook Messenger corroborates descriptions in the literature of what the most popular mobile messenger apps within the field of medicine are, worldwide.[12-15] Since the 1990s, these technologies have changed daily human activities, not only individually or economically, but also socially. A notable percentage of the participants stated that they used apps for medical issues frequently (daily, weekly or monthly), which demonstrates the applicability of such tools. As described by Giodano et al., these apps are an ideal tool for quick reference, as well as for clinical, academic and propaedeutic endorsements or for communication between healthcare professionals and patients, because of the inherent characteristics of mobile apps.[16]

The effect of using a secure messaging app (WhatsApp) for medical consultations in an emergency department was assessed in a randomized controlled study. Comparisons were made with consultation conducted by telephone. It was shown that use of the app (i.e. the intervention group) reduced the median length of stay in the emergency department (240 minutes versus 277 minutes) and reduced the median time spent on consultations (158 minutes versus 170 minutes).[17]

A British study assessed the implementation of the WhatsApp service within emergency surgical teams, in which the team members (n = 40) exclusively used WhatsApp for 19 weeks. It was demonstrated that use of this instant messaging tool promoted better communication of instructions, faster communication between interns and for attendance, and a flattened hierarchy among the team members.[18]

Another important study, conducted within the field of cardiovascular medicine, assessed the efficacy of WhatsApp for attending cases of ST-segment elevation myocardial infarction (n = 108) in rural areas in Turkey. It was observed that use of this app had a positive impact on triage and early activation of the cardiac catheterization laboratory, reduced door-to-balloon time and was an approach in keeping with international guidelines.

Thus, from these different reports, it can be seen that instant messaging apps are an efficient communication tool that enables resolution of problems within different medical specialties.

Regarding the legal aspects of use of IMS apps, our study showed that around 63% of the participants did not have any legal knowledge or had pursued non-literature-based knowledge in relation to the use of instant messaging apps for case discussions, imaging sharing and patient guidance.

In Brazil, even though more than three decades have gone by since the initial experiences with telehealth, legislative codification of telemedicine services into law, along with regulatory policies, remain at an early stage and information regarding this has not been disseminated among healthcare professionals. In contrast, in the United States and Germany, the use of telehealth services is underpinned by laws and regulations, thus resulting in bettering of medical practice and physician-patient relationships, given that the pre-specified policies avoid further prosecution and ethical concerns. Therefore, it is likely that thorough analysis of the legislature around the world is required in order to adapt and implement all the relevant medical jurisprudences for the scenario in one specific state in Brazil.

Medical practice is also constantly influenced by the approaches provided through instant messaging apps. The respondents in the present study perceived that the impact may be either positive or negative. General concerns relating to data protection and privacy are certainly relevant, and these ought to legitimate regulative intervention to avoid misuse and medico-ethical issues.

In agreement with previous studies, we observed that instant messaging services are an alternative way for physicians to communicate with patients or their families, and that these services present several advantages.[24-26] The perceived benefits that have been observed within medical practice include reduction of medical errors[31] due to rapid online consultation and information sharing; strengthening of physician-patient relationships, related to creation of "dedicated conversation channels"; democratization of medical management,[21,22] since team-based decision-making can be implemented and patients' preferences can also be considered; and most importantly, increased access to healthcare services, through reaching out to remote and socially vulnerable populations.[23]

In a Malaysian study, it was perceived that use of mobile messaging apps had a positive effect regarding coronary artery disease patients' knowledge of and adherence to a healthy lifestyle. It was concluded that such tools, specifically WhatsApp, are useful additional mechanisms within current medical practice.[27] Another previous randomized controlled trial assessing the use of instant messaging software for following up patients who were undergoing peritoneal dialysis showed that there was a higher degree of satisfaction among those using online approaches than among those in the traditional group.[28]

Strengths and limitations of this study

This study raises awareness regarding the necessity for both legal and medical regulations for the use of instant messaging within medical practice. It is a pioneer in that it not only demonstrates the frequency and types of use of the main application (WhatsApp) but also demonstrates the use of other equivalent platforms.

The main limitation of the present study was in relation to the data collection process. Most of our participants were medical students, and this may have been a bias factor, given that when they were in contact with patients they would preferably be under the responsibility and supervision of a medical professor, to guide their clinical approach and management, which could have impacted on the participants' behavior. Our research group tried to obtain support from the Brazilian national board of physicians to expand the survey nationwide among medical doctors, but this has not been possible so far.

Furthermore, the survey link was sent through an outsourcing procedure on the University website. This did not allow access to information on the exact number of people who received the email but did not even open it and the number who did open it.

Also, the study was based on self-reported data, for which the rate of return of responses tends to be lower. It was impossible to assess how representative our sample was, in relation to the entire number of people who opened the email.

On the other hand, the use of a self-reported questionnaire may have been a strength, given that there was no interviewer creating bias through selection of answers and that the responders had autonomy.

CONCLUSION

This study demonstrated how popular instant messaging apps have become among physicians and medical students nowadays, following the global trend. However, at the same time, it demonstrated how little both physicians and medical students know about the legal implications of the use of these tools. Therefore, it is advisable that regulatory legislation should be brought forward. Moreover, groundbreaking standard operating procedures should be proposed in order to ensure safety and security for all parties involved (physicians, patients, medical students and so forth). This is especially needed in developing countries such as Brazil, where regulations on this matter remain scarce.

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It should read:

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Typical main headings in the text include Introduction, Methods, Results, Discussion and Conclusion. The authors can and should use short subheadings too, especially those concerning the reporting guideline items.

Trial and systematic review registration policy

São Paulo Medical Journal supports the clinical trial registration policies of the World Health Organization (WHO) and the International Committee of Medical Journal Editors (ICMJE) and recognizes the importance of these initiatives for registration and international dissemination of information on randomized clinical trials, with open access. Thus, since 2008, manuscripts on clinical trials are accepted for publication if they have received an identification number from one of the public clinical trial registration database (such as ClinicalTrials.gov and/or REBEC and/or the World Health Organization; the options are stated at http://www.icmje.org). The identification number should be declared at the end of the abstract. Articles describing systematic reviews must provide the protocol registration number in the PROSPERO database. Articles presenting clinical trials or systematic reviews without registration protocols will be promptly rejected without peer review.

Results from cases with DNA sequences must be deposited in appropriate public databases. The protocol number or URL can be requested at any time during the editorial review. Publication of other research data in public repositories is also recommended, since it contributes towards replicability of research, increases article visibility and possibly improves access to health information.

Sample size

All studies published in SPMJ must present a description of how the sample size was arrived at. If it was a convenience or purposive sample, the authors must declare so and explain the characteristics of this sample and recruitment method. For clinical trials, for instance, it is mandatory to inform each of the three main values used to calculate sample size:

- power (usually 80% or more);
- level of significance (usually 0.05 or lower);
- clinically meaningful difference (effect size targeted), according to the main outcome measurement.

Regardless of study results (if "positive" or "negative"), the journal will probably reject articles of trials using underpowered samples, when sample size has not been properly calculated or the calculation has not been fully described as indicated above.

Abbreviations, acronyms and products

Abbreviations and acronyms must not be used, even those in everyday use, unless they are defined when first used in the text. However, authors should avoid them for clarity whenever possible. Drugs or medications must be referred to using their generic names (without capital letters), with avoidance of casual mention of commercial or brand names.

Interventions

All drugs, including anesthetics, should be followed by the dosage and posology used.

Any product cited in the Methods section, such as diagnostic or therapeutic equipment, tests, reagents, instruments, utensils, prostheses, orthoses and intraoperative devices, must be described together with the manufacturer's name and place (city and country) of manufacture in parentheses. The version of the software used should be mentioned.

Any other interventions, such as exercises, psychological assessments or educational sessions, should be described in enough details to allow reproducibility. The Journal recommends that the TIDieR reporting guidelines should be used to describe interventions, both in clinical trials and in observational studies.[13]

Short communications

Short communications are reports on the results from ongoing studies or studies that have recently been concluded for which urgent publication is important. They should be structured in the same way as original articles. The authors of this kind of communication should explain, in the covering letter, why they believe that publication is urgent. Short communications and case reports must be limited to 1,000 words (from the introduction to the end of the conclusion).

Case reports, case series, narrative reviews and letters to the editor

Starting in June 2018, only individual case reports dealing with situations of public health emergencies will be accepted by *São Paulo Medical Journal*. Case reports that had already been accepted for publication up to May 2018 will still be published in a timely manner.

After initial evaluation of scope by the editor-in-chief, case reports, case series and narrative reviews will be considered for peer-review evaluation only when accompanied by a systematic search of the literature, in which relevant studies found (based on their level of evidence) are presented and discussed.[12] The search strategy for each database and the number of articles obtained from each database should be shown in a table. This is mandatory for all case reports, case series and narrative reviews submitted for publication. Failure to provide the search description will lead to rejection before peer review.

The access route to the electronic databases used should be stated (for example, PubMed, OVID, Elsevier or Bireme). For the search strategies, MeSH terms must be used for Medline, LILACS, and Cochrane Library. DeCS terms must be used for LILACS. EMTREE terms must be used for Embase. Also, for LILACS, the search strategy must be conducted using English (MeSH), Spanish (DeCS) and Portuguese (DeCS) terms concomitantly. The search strategies must be presented exactly as they were used during the search, including parentheses, quotation marks and Boolean operators (AND, OR, and NOT). The search dates should be indicated in the text or in the table.

Patients have the right to privacy. Submission of case reports and case series must contain a declaration that all patients gave their consent to have their cases reported (even for patients cared for in public institutions), in text and images (photographs or imaging examination reproductions). The Journal will take care to cover any anatomical part or examination section that might allow patient identification. For deceased patients whose relatives cannot be contacted, the authors should consult the Editor-in-Chief. All case reports and case series must be evaluated and approved by an ethics committee.

Case reports should be reported in accordance with the CARE Statement,[7] including a timeline of interventions. They should be structured in the same way as original articles.

Case reports must not be submitted as letters. Letters to the editor address articles that have been published in the *São Paulo Medical Journal* or may deal with health issues of interest. In the category of letters to the editor, the text has a free format, but must not exceed 500 words and five references.

FORMAT: FOR ALL TYPES OF ARTICLES

Title page

The title page must contain the following items:

1. Type of paper (original article, review or updating article, short communication or letter to the editor);
2. Title of the paper in English, which should be brief but informative, and should mention the study design.[14] Clinical trial, cohort, cross-sectional or case-control study, and systematic review are the most common study designs. Note: the study design declared in the title should be the same in the methods and in the abstract;
3. Full name of each author. The editorial policy of the *São Paulo Medical Journal* is that abbreviations of authors' names must not be used; therefore, we ask that names be stated in full, without using abbreviations;
4. Each author should present his/her ORCID identification number (as obtained from www.orcid.org);
5. Each author should indicate the way his/her name should be used in indexing. For example: for "João Costa Andrade", the indexed name could be "Costa-Andrade J." or "Andrade JC", as preferred;
6. Each author should indicate a valid, up-to-date email address for contact;
7. The author's professional background (Physician, Pharmacist, Nurse, Dietitian or another professional description, or Undergraduate Student); and his/her position currently held (for example, Master's or Doctoral Student, Assistant Professor, Associate Professor or Professor), in the department and institution where he/she works, and the city and country (affiliations);
8. Place or institution where the work was developed, city and country.
9. Date and venue of the event at which the paper was presented, if applicable, such as congresses, seminars or dissertation or thesis presentations.
10. Sources of financial support for the study, bursaries or funding for purchasing or donation of equipment or drugs. The protocol number for the funding must be presented with the name of the issuing institution. For Brazilian authors, all grants that can be considered to be related to production of the study must be declared, such as fellowships for undergraduate, master's and doctoral students; along with possible support for postgraduate programs (such as CAPES) and for the authors individually, such as awards for established investigators (productivity; CNPq), accompanied by the respective grant numbers.
11. Description of any conflicts of interest held by the authors (see above).
12. Complete postal address, e-mail address and telephone number of the author to be contacted about the publication process in the Journal (the "corresponding author"). This author should also indicate a postal address, e-mail address and telephone number that can be published together with the article. *São Paulo Medical Journal* recommends that an office address (rather than a residential address) should be informed for publication.

Second page: abstract and keywords

The second page must include the title and a structured abstract in English with a maximum of 250 words. References must not be cited in the abstract.

The following headings must be used in the structured abstract:

- Background – Describe the context and rationale for the study;
- Objectives - Describe the study aims. These aims need to be concordant with the study objectives in the main text of the article, and with the conclusions;
- Design and setting – Declare the study design correctly, and the setting (type of institution or center and geographical location);
- Methods – Describe the methods briefly. It is not necessary to give all the details on statistics in the abstract;
- Results – Report the primary results;
- Conclusions – Make a succinct statement about data interpretation, answering the research question presented previously. Check that this is concordant with the conclusions in the main text of the article;
- Clinical Trial or Systematic Review Registration – Mandatory for clinical trials and systematic reviews; optional for observational studies. List the URL, as well as the Unique Identifier, on the publicly accessible website on which the trial is registered.
- MeSH Terms - Three to five keywords in English must be chosen from the Medical Subject Headings (MeSH) list of Index Medicus, which is available at http://www.ncbi.nlm.nih.gov/sites/entrez?db=mesh. These terms will help librarians to quickly index the article.
- Author keywords - The authors should also add three to six "author keywords" that they think express the main article themes. These keywords should be different from the MeSH terms and preferably different from words already used in the title and abstract, so as to improve the discoverability of the article by readers doing a search in PubMed. They provide an additional chance for the article to be retrieved, read and cited. Combinations of words and variations (different wording or plurals, for example) are encouraged.

References

For any manuscript, all statements in the text that do not result from the study presented for publication in the *São Paulo Medical Journal* but from other studies must be accompanied by a quotation of the source of the data. All statements regarding health statistics and epidemiological data should generally be followed by references to the sources that generated this information, even if the data are only available electronically.

São Paulo Medical Journal uses the reference style known as the "Vancouver style," as recommended by the International Committee of Medical Journal Editors (ICMJE). Follow the instructions and examples at www.icmje.org, item "References", for the format.

In the text, the references must be numbered in the order of citation. The citation numbers must be inserted after periods/full stops or commas in sentences, and in superscript (without parentheses or square brackets). References cited in the legends of tables and figures must maintain sequence with the references mentioned in the text.

In the list of references, all the authors must be listed if there are up to and including five authors; if there are six or more, the first three should be cited, followed by the expression "et al." For books, the city of publication and the name of the publishing house are mandatory. For texts published on the internet, the complete uniform resource locator (URL) or address is necessary (not only the main home page of a website or link), so that by copying the complete address into a computer internet browser, the Journal's readers will be taken to the exact document cited, and not to a general website.

At the end of each reference, please insert the "PMID" number (for papers indexed in PubMed) and the "doi" number if available.

Authors are responsible for providing a complete and accurate list of references. All references cited in the text must appear in the reference list, and every item in the reference list must be cited in the text. Also, citations must be in the correct sequence.

Manuscripts that do not follow these guidelines for references will be returned to the authors for adjustments.

The reference list should be inserted after the conclusions and before the tables and figures.

Figures and tables

Images must be submitted at a minimum size that is reproducible in the printed edition. Figures should be sent at a resolution of 300 DPI and minimum size of 2,500 pixels (width) and be recorded in ".jpg" or ".tif" format. Images submitted in inadequate formats will not be accepted.

Images must not be embedded inside Microsoft PowerPoint or Microsoft Word documents, because this reduces the image size. Authors must send the images separately, outside of .doc or .ppt documents. Failure to send the original images at appropriate sizes leads to paper rejection before peer review.

Flowcharts are an exception: these must be drawn in an editable document (such as Microsoft Word or PowerPoint), and should not be sent as an image that can't be changed.

Figures such as bars of line graphs should be accompanied by the tables of data from which they have been generated (for example, sending them in the Microsoft Excel spreadsheets, and not as image files). This allows the Journal to correct legends and titles if necessary, and to format the graphs according to the Journal's style. Graphs generated from software such as SPSS or RevMan must be generated at the appropriate size, so that they can be printed (see above). Authors must provide internal legends/captions in correct English.

All the figures and tables should be cited in the text. All figures and tables must contain legends or titles that precisely describe their content and the context or sample from which the information was obtained (i.e. what the results presented are and what the kind of

sample or setting was). The reader should be able to understand the content of the figures and tables simply by reading the titles (without the need to consult the text), i.e. titles should be complete. Acronyms or abbreviations in figure and table titles are not acceptable. If it is necessary to use acronyms or abbreviations inside a table or figure (for better formatting), they must be spelled out in a legend below the table or figure.

For figures relating to microscopic findings (i.e. histopathological results), a scale must be embedded in the image to indicate the magnification used (just like in a map scale). The staining agents (in histology or immunohistochemistry evaluations) should be specified in the figure legend.

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