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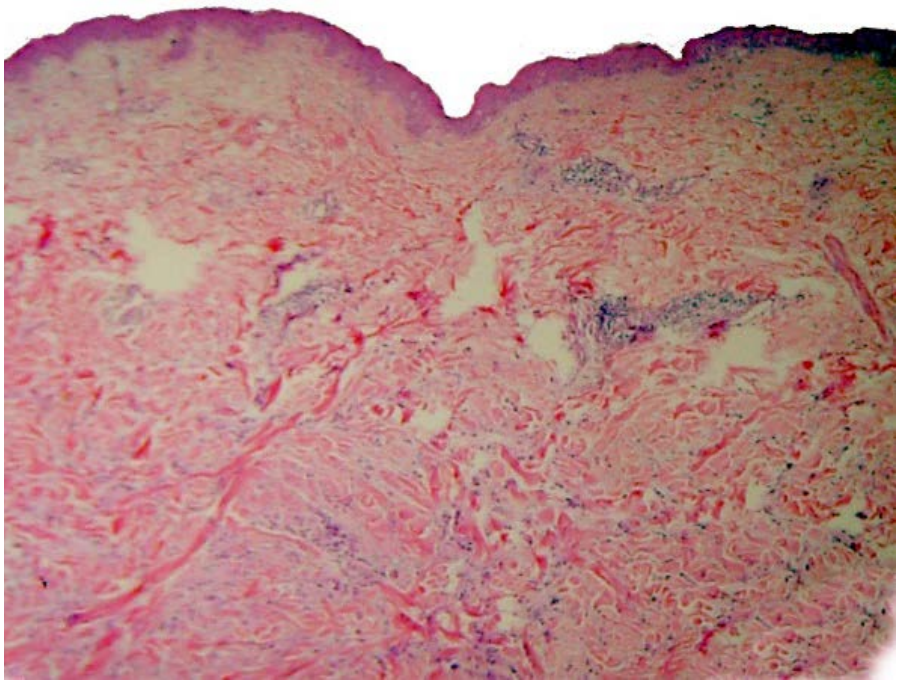

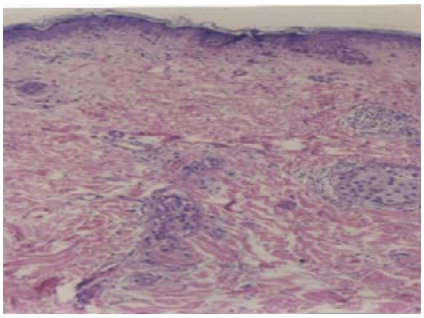

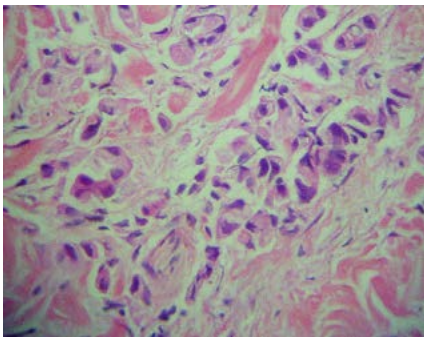

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ARTICLES

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JOURNAL OF THE BRAZILIAN MEDICAL ASSOCIATION

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SUMMARY

SECTIONS

ARTICLES

The brazilian FRAX model: an introduction

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Osteoporosis is a systemic skeletal disease characterized by low bone mass and deterioration of bone microarchitecture leading to fractures secondary to minimal trauma. Vertebra, hip and forearm are common sites of these fragility fractures. The frequent association between fragility fractures and an increase in morbidity and mortality makes osteoporosis a huge socioeconomic and public health burden for many countries. This chronic bone disorder affects mainly postmenopausal women but can also develop in older man. It is calculated that at the age of 50 years, the lifetime fracture risk is 50% for women and 25% for men [1].

Predictions based on epidemiological studies reveal a continuous increase in the world's population, probably reaching approximately 7.5 to 10.5 billion people by the year 2050. It is noteworthy that Latin America and the Caribbean region account for 9% of this global population. Brazil, the biggest country in Latin American (LATAM), is the fifth most populous country in the world and accounts for 32% of the individuals in this region. The International Osteoporosis Foundation (IOF) published, in 2012[2], a large epidemiological report – the Latin America Regional Audit – that gathered information from a literature search and/or provided by key opinion leaders on the burden of osteoporosis in 14 countries from Latin America. Firstly, this Audit showed that, although the current percentage of people 50 years of age and older lies between 13 and 29% in those countries, it is estimated that by 2050 these figures will be 28 to 49 % with a 280% increase in the 70 and over population. The aging of these populations is a matter of concern due to the many diseases that mainly affect older people, including osteoporosis and its related fragility fractures. In LATAM, the current data on osteopenia and osteoporosis is scarce, but some studies bring good epidemiological information. Based on these, models for the FRAX calculation tool regarding the absolute risk for fragility fractures were constructed for 6 countries in LATAM: Argentine, Brazil, Chile, Colombia, Ecuador and Mexico.

FRAX® is a computer-based algorithm developed by the Centre for Metabolic Bone Diseases, University of Sheffield Medical School, UK, first released in 2008 (http://www.shef.ac.uk/FRAX) [3,4]. The algo-

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rithm, calculates the 10-year probability of a major fracture (hip, clinical spine, humerus, or wrist) and the 10-year probability of hip fracture in postmenopausal women and men aged 50 years or older.

Fracture risk is readily calculated from age, body mass index (BMI) and dichotomized (yes or no) clinical risk factors (CRFs) comprising prior fragility, parenteral history of hip fracture, current tobacco smoking, long-term oral glucocorticoid use, rheumatoid arthritis, other causes of secondary osteoporosis, and alcohol consumption. Rheumatoid arthritis and long-term use of glucocorticoids are risk factors independent of their action on BMD, the other secondary causes of osteoporosis considered in the algorithm are assumed to influence the increased risk for fracture by their lowering of BMD.

The bone mineral density (BMD) of the femoral neck can be optionally entered to enhance fracture risk prediction, but the FRAX algorithm output can be calculated without this information. If available, the inclusion of BMD in the calculation of fracture probability improves the accuracy of the assessment but it is most needed in individuals in whom fracture probabilities lie close to an intervention threshold. This is defined as the fracture probability at which physicians may intervene.

Fracture probability differs greatly in different parts of the world [5], and the FRAX calibration has been made individually for each country where the epidemiology of hip fracture and death is published. The FRAX model is unique because unlike other algorithms, fracture probability is computed by taking the risk of fracture and the risk of death into account. The inclusion of risk of death is important because individuals with an immediate probability of death are less likely to suffer from fractures than those with longer life expectancy. In addition, some risk factors affect the risk of death as well as the risk of fracture. Examples include increasing age, low BMI, low BMD, long-term use of glucocorticoids, and smoking.

The association between risk factors and fracture risk has been constructed using information derived from large primary data of population-based cohorts from many countries around the world [6,7,8]. A series of meta-analyses based on those international cohorts identified clinical risk factors for fracture that provided independent information on fracture risk [4]. The relationship between risk factors for fracture included in the FRAX algorithm do not differ significantly among different countries and can be universally used taking into account the available evidence.

FRAX models are currently available for 63 countries and 32 languages covering 79% of the world population aged 50 years or more. Ethnic-specific models are available only in the U.S. and Singapore. The FRAX model for Brazil has been internet launched in 2013 and the description of its construction with the calculation of the national incidence of hip fracture in Brazil became available in 2015 [9].

Brazil has a population of 207,690,929 inhabitants living mainly in urban areas. The country has 26 million people aged 60 years and older comprising 12.5% of the population, and this percentage will rise to 37.9 million in 2017. The country is divided into five regions, but the majority of the population lives in the Southeast (42,13%), Northeast (27.83 %), and South (14.36%). [10]

Data from four Brazilian epidemiologic studies (table below) were collected and analyzed to obtain national data on the incidence of hip fracture and mortality [11-14]. These studies have been conducted in the cities of Porto Alegre located in the South [11], Marilia in the Southeast [12] and Sobral and Fortaleza in the Northeast [13,14] regions of the country.

The studies from Porto Alegre, Marilia, and Sobral were retrospective and the Fortaleza study was prospective.

The development and validation of the Brazilian FRAX model followed the method universally used for this tool [3,4]. The risk factors used in the Brazilian model were based on a systematic set of meta-analyses of worldwide population-based cohorts and validated in independent cohorts with over a million patient-years of follow-up (please see reference 9 for more information).

For the clinicians, FRAX provides a quantitative estimate for fracture risk and, thereby eliminates the

POPULATION AT RISK AND ANNUAL HIP FRACTURE RATES BY AGE AND SEX IN THE FOUR BRAZILIAN STUDIES

Age	Fractures		Population		Incidence per 100, 000	
	Men	Women	Men	Women	Men	Women
40-49	14	10.5	68196	83471	21	13
50-59	29.8	44.8	126376	160252	24	29
60-69	52.6	88.8	79423	111568	66	80
70-79	47.6	223.2	34592	57286	138	390
80-89	21.2	113.9	3424	9020	618	1263
90+	3.4	21.5	297	953	1144	2252

uncertainty of an individual's practitioner qualitative assessment of risk.

Regarding the intervention thresholds, the approach recommended by the National Osteoporosis Guideline Group (NOGG) in the UK [15,16] was used in the Brazilian FRAX model. This methodology sets the intervention threshold at the age-specific fracture probability equivalent to women (or men) with a prior fragility fracture. Where access to BMD testing is limited, FRAX can be calculated using BMI and the use of BMD can be optimized by only testing those individuals in whom probabilities are close to the intervention threshold [15,17,18]. In this way, testing is confined to individuals at high (or low) risk with reasonable likelihood to be reclassified at low (or high) risk on the basis of the BMD test. Following this approach, two assessment thresholds were calculated and applied to the intervention threshold described above:

The threshold probability below which neither treatment nor a BMD test should be considered (lower assessment threshold).

The threshold probability above which treatment may be recommended without the need for BMD (upper assessment threshold).

The results of this calculation were displayed in figures showing the fracture probabilities equivalent to women (or men) with a previous fragility fracture in the FRAX Brazil model. These figures will be soon available for clinical use in the website of the Brazilian Medical Association.

FRAX represents a significant advance in the assessment of both women and men at risk of osteoporosis-related fractures and allows the tailoring of pharmacological interventions to high-risk subjects. However, it has limitations and must be used only as a guideline. The practitioner clinical judgment will, and should, supplant any calculated value. Furthermore, it is a tool in evolution, being refined as the databases are updated with more epidemiological information.

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Spinal muscular atrophy 5Q – Treatment with nusinersen

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

The spinal muscular atrophy (SMA) is a neurodegenerative condition with autosomal recessive genetic inheritance. Nusinersen is an antisense oligonucleotide drug that modifies the SMN2 pre-mRNA processing to promote increased production of the full-length SMN protein. The purpose of this guideline is to provide recommendations that may assist in the decision-making regarding the use of nusinersen in patients with SMA 5q. For this, a systematic review of the literature was performed, without period restriction, in the Medline/PubMed, Central (Cochrane), and Lilacs databases via VHL, retrieving 243 papers, of which two randomized clinical trials were selected to respond to clinical doubt. The details about the methodology and the results are set out in Appendix I.

INTRODUCTION

The spinal muscular atrophy (SMA) is a neurodegenerative condition with autosomal recessive genetic inheritance. It is caused by a homozygous deletion of the survival motor neuron gene (SMN1). This genetic alteration results in a reduction of survival motor neuron (SMN) protein levels, leading to spinal cord alpha motor neurons degeneration, resulting in progressive symmetric proximal muscle weakness and paralysis[1,2]. Nusinersen is an antisense oligonucleotide drug that modifies the SMN2 pre-mRNA processing to promote increased production of the full-length SMN protein[3].

The incidence of SMA is often cited as approximately ten in every 100,000 live births. A recent review found estimates ranging from 5.0 to 24 per 100,000 births. The estimated prevalence is approximately one to two in 100,000 people[4].

SMA is diagnosed through genetic testing. An initial test evaluates the homozygous deletion of 5q in the survival motor neuron 1 (SMN1) gene, which identifies 95% of cases. If negative, the sequencing of the SMN1 gene is carried out as a second step. Nerve conduction studies and electromyography (EMG) are performed in a subgroup of patients. However, even when evidence of motor neuronopathy is identified in the study, a confirmatory genetic testing is carried out[5,6].

They are classified as type I (Werdnig-Hoffman disease), type II (Dubowitz disease), Type III (Kugelberg-Welander disease) and type IV (adult form). Type I is fatal in childhood, type II has a late onset during childhood and is associated with survival up to the second or third decade. Type III begins in childhood, is slowly progressive and comprises about 10% to 20% of all patients with SMA[7]. SMA type IV is the adult phenotype of SMA, characterized by mild muscle weakness usually beginning in the second or third decade of life. Infants with onset of symptoms during the prenatal period or within the first week of life are classified with SMA type 0, a very rare phenotype (<1%)[9].

SMA Type III: (also called juvenile SMA or Kugelberg-Welander disease), it appears after 18 months, but the age of onset varies greatly. According to Wirth et al.[8], the onset of the disease before 3 years of age is classified as SMA type IIIa, whereas, after this age, it is classified as SMA type IIIb. What differentiates both is the ability to walk, with individuals with type IIIa being able to walk up to the age of 20, while type IIIb patients of the same age never lose that ability[9]. Difficulties in swallowing, coughing, or nocturnal hypoventilation are less frequent than in type II patients, but they may occur. Over the years, these individuals may develop scoliosis. The life expectancy for these patients is undefined[10].

RESULTS

The Endear[3] study (Finkel, L. et al., 2017) assessed children who had genetic documentation of a homozygous deletion or mutation in the SMN1 gene; two copies of the SMN2 gene and, therefore, is considered more likely to develop type I SMA; onset of clinical symptoms compatible with spinal muscular atrophy at 6 months of age or younger; were 7 months of age or younger at screening and did not have low peripheral oxygen saturation (ie, did not require respiratory care). Exclusion criteria for this study were patients with hypoxemia, signs or symptoms of SMA present at birth or in the first week after birth, history or active condition that would interfere with lumbar puncture or study evaluation, and any history of gene therapy, prior antisense oligonucleotide (ASO) or cell transplantation.

Randomization was stratified according to the duration of the disease. The intervention was the intrathecal administration of nusinersen (nusinersen group) at an adjusted dose according to the estimated volume of cerebrospinal fluid for age, in such way that a patient of 2 years of age or more received the equivalent of a 12 mg dose (in a 5 ml solution), and younger children received smaller volumes, containing smaller doses of the drug. In the nusinersen group, doses were given on days 1, 15, 29 and 64, and maintenance doses on days 183 and 302 (maintenance dose every four months). A sham procedure[3] was used on the control group (**A**). **Table 1**

Prognostic differences in this study: patients treated with nusinersen at the beginning of the study had a higher percentage of paradoxical breathing (89% vs 66%), pneumonia or respiratory symptoms (35% vs 22%), difficulties in swallowing or feeding (51% vs 29%) and need of respiratory support (26% vs 15%) compared with patients in the sham group.

A pre-specified interim analysis was conducted by the sponsor and the data and safety monitoring board in which approximately 80 children were enrolled for at least six months. The analysis showed a benefit-risk assessment in favor of nusinersen. This result led to the early termination of the study. At that time, children were invited to undergo an end-of-study visit at least two weeks after receiving their most recent dose of nusinersen or having undergone their most recent dummy procedure.

By the end date of the final analysis, 39% of the nusinersen and 68% of the control group babies died or received permanent ventilatory support (event-free survival[c])[3] (**A**).

The composite outcome death OR permanent ventilatory support use had a likelihood of occurrence, at any point in time, 47% lower in the nusin-

TABLE 1 - BENEFIT AND/OR HARM - ABSOLUTE DATA

OUTCOME	N/NEC	N/NEI	ARC%	ARI%	IAR% (95%CI)	NNT	95%CI
HINE respondents Section 2 (6-month interim analysis)	27/0	51/21	0	41.2	41.2 (27.7 - 54.7)	2	2 - 4
Respondents CHOP INTEND [b]	37/1	73/52	3%	71%	68.5 (57 - 80)	1	1 - 2
Adverse events	41/40	80/77	97.6	96.3	1.3 -4.9 - 7.5	NS	

N: number of patients analyzed; NEI: number of events in intervention; **NEC**: number of events in control; ARI: absolute risk in intervention; **ARC**: absolute risk in comparison; **ARR**: absolute risk reduction; **IAR**: increase in absolute risk; **NNT**: Number needed to treat; **NNH**: number needed to harm; **CI:** confidence interval of 95%; **ITT**: analysis by intention to treat. (**a**) Respondent of Hine section 2 = According to the section 2 of the Hammersmith Infant Neurological Examination - Hine: an increase of ≥2 points [or maximum score] in the ability to kick, OR an increase ≥1 point in the motor control steps of the head, roll, sit, crawl, stand or walk, and improvements in more categories of motor stages than aggravations is defined as a respondent for this primary analysis. (**b**)Respondent of Chop Intend = percentage of patients with at least 4-point improvement over baseline in the Children's Hospital of Philadelphia Infant Test for Neuromuscular Disease - Chop Intend - whose scores range from 0 to 64, with the highest scores indicating better motor function. (**c**) Event-free survival = Event-free survival, which was defined as the time up to death or use of permanent assisted ventilation (tracheostomy or ventilatory support for ≥16 hours per day for >21 continuous days, in the absence of an acute reversible event).

ersen-treated group (Hazard Ratio (HR) =0.53; 95% Confidence Interval [CI], 0.32-0.89, p=0.005). This benefit was higher among patients included in the study with disease duration ≤13.1 months, compared with those with >13.1 months[3] **(A)**.

The median time until death or use of permanent ventilatory support was 22.6 weeks in the control group and was not achieved in the nusinersen group[3] **(A)**.

When results were separated for each type of outcome (death and permanent ventilatory support), the results indicated a statistically significant difference between the nusinersen group and the simulated procedure in overall survival (HR=0.37, 95%CI 0.18 to 0 , 77), but not for permanent ventilatory support (HR=0.66, 95%CI 0.32 to 1.37). It is possible, however, that due to loss of data caused by the premature termination of the study, as well as a shorter duration of follow-up, the statistical power has been reduced[3] **(A)**.

A smaller percentage of infants in the nusinersen group than in the control group died at the end of the study (16% vs 39%). The death outcome had a likelihood of occurrence, at any point in time, 63% lower in the nusinersen-treated group (HR=0.37; 95%CI, 0.18 to 0.77; p=0.004). There was no difference between groups in the likelihood of using permanent ventilatory support at any point in time (HR=0.66 95% CI (0.32-1.37); p=0.13); 23% of the children in the nusinersen group and 32% in the control group received permanent ventilatory support)[3] **(A)**.

RECOMMENDATION

In children with a diagnosis of SMA type I, the use of intrathecal nusinersen with a dose adjusted according to the estimated volume of cerebrospinal fluid by age (equivalent to a dose of 12 mg for a 2-year-old patient) given on days 1, 15, 29 and 64 and maintenance doses on days 183 and 302 (maintenance doses every four months), compared to a simulated treatment, in up to six months:

- Increases the number of "respondent" patients (with improved motor function) by 41.2%, being necessary to treat two patients so that one was "respondent" (NNT = 2) - analysis with Hine section 2. Study power for bilateral 95% IC is 98%. In an intention-to-treat analysis (ITT), the number of "respondents" increased by 26%, 95%CI 17 to 36; being necessary to treat four patients for every "respondent" (NNT = 4, 95%CI 3 to 6), with a study power for bilateral 95%CI of 95.7%. **(A)** **(Table 1)**
- The outcome death OR permanent ventilatory support use (composite outcome) had a likelihood of occurrence, at any point in time, 47% lower in the nusinersen-treated group. This benefit was higher among patients with disease duration ≤13.1 months. **(A)**
- The death outcome had a likelihood of occurrence, at any point in time, 63% lower in the nusinersen-treated group. **(A)**
- There is no difference between groups in the likelihood of using permanent ventilatory support at any point in time. **(A)**
- The proportion of patients who achieve an improvement of 4 or more points ("respondents") increases by 68% in the Children's Hospital of Philadelphia Infant Test for Neuromuscular Disease - Chop Intend, whose scores range from 0 to 64, and higher scores indicate better motor function (NNT = 1). Study power for bilateral 95% IC is 100%. **(A)** **(Table 1)**
- There is no difference in the number of treatment-related adverse events between both groups. **(A)**

The Cherish[11] (Mercuri, E. et al., 2018) randomized phase III study, sham-controlled, included patients (N=126) with symptoms compatible with SMA type II and age between 2 and 12 years (84% of patients at baseline were under 6 years of age)[11](A). Patients presented genetic documentation of deletion of the homozygous 5q SMA gene, homozygous or composite heterozygous mutation, and beginning of clinical signs and SMA-compatible symptoms after 6 months of age. They could sit independently but never had the ability to walk independently. They had a Hammersmith Functional Motor Scale-Expanded (HFMSE) score for motor function of ≥10 and ≤54 at screening (HFMSE scores range from 0 to 66, with higher scores indicating better motor function). The following exclusion criteria were considered: respiratory failure, gastroenteric tube feeding, severe scoliosis and contractures, history or active condition that would interfere with lumbar puncture, treatment with another experimental drug, treatment with valproate or hydroxyurea in the last three months, any history of gene therapy, antisense oligonucleotide therapy, or cell transplantation.

The intervention group (n=84) received 12 mg (in a 5 mL solution) of nusinersen administered intrathecally on days 1, 29, 85 and 274 (maintenance dose every six months) and the control group (n=42), a simulated procedure (sham group)[11] **(A)**.

Prognostic differences in this study: an imbalance in the proportion of patients who had been able to stand up unsupported (13% of patients in the nusinergen group, 29% in the sham control group) or walk with support (24% of patients in the nusinergen group and 33% in the control group).

The Cherish study was prematurely terminated due to ethical reasons arising out of the positive results generated from an interim analysis.

The interim analysis of the primary outcome was performed when all the children had been enrolled for at least six months, and at least 39 children completed the evaluation of 15 months. The analysis was performed with the use of a multiple imputation method. The number of children with data observed for the 15-month evaluation was 35 in the nusinersen group and 19 in the control group, and the number of children with imputed data was 49 in the nusinersen group and 23 in the control group. In the final analysis, the following outcomes were analyzed using a multiple imputation method: baseline change in the HFMSE score, percentage of children with a change in HFMSE score of at least 3 points, and baseline change in the Revised Upper Limb Module (Rulm) ranging from 0 to 37, with higher scores indicating better motor function. The percentage of children who achieved at least one new World Health Organization (WHO) milestone (out of a total of six milestones) was also assessed.

Only children with observed data were included in the other analyzes. The number of children with data observed for the 15-month evaluation was 66 in the nusinersen group and 34 in the control group, and the number of children with imputed data was 18 in the nusinersen group and 8 in the control group[11](A).

There was improvement in motor function (HFMSE score) from the start of the study in nusinersen-treated patients compared to control patients (difference in minimum mean square points, 5.9 (3.7 to 8.1); p <0.0001). HFMSE scores range from 0 to 66, with higher scores indicating better motor function[11](**A**). (**Table 2**)

There was an improvement in motor function from the baseline in the Rulm score (ranging from 0 to 37, with higher scores indicating better motor function) with the use of nusinersen in comparison with the control group (difference of minimum mean square points 3.7 (2.3 to 5.0), p <0.0001)[11](**A**). (**Table 2**)

A higher percentage of children in the nusinersen group compared to the control one had a baseline increase, at month 15 in the HFMSE score, of at least 3 points (57% vs 26%, P <0.001)[11](**A**).

The percentage of children who achieved at least one new WHO milestone did not differ significantly between the nusinersen group and the sham group (20% [95% CI 11 to 31] and 6% [CI 95% 1 to 20], respectively; 14% ratio difference [-7 to 34], p=0.08)[11](**A**).

The overall incidence of adverse events was similar in the nusinersen and control groups (93% and 100%, respectively), as well as the incidence of moderate or severe adverse events[11](**A**).

RECOMMENDATION

In children with a diagnosis of SMA type II, the use of intrathecal nusinersen at a 12 mg dose (in a 5 ml solution) administered on days 1, 29, 85 and 274 (maintenance dose every six months), in up to 15 months:

- Improves motor function (HFMSE score) - difference in minimum mean square points = 5.9 (3.7 to 8.1), p<0.0001). HFMSE scores range from 0 to 66, with higher scores indicating better motor function. (**A**)
- Increases baseline HFMSE score in at least 3 points (HFMSE scores range from 0 to 66, with higher scores indicating better motor function), (57% vs 26%, p<0.001). (**A**)
- There is no difference in the percentage of children who achieved at least one new WHO milestone, out of a total of six milestones. (**A**)
- Improves motor function from the baseline in the Rulm score (ranging from 0 to 37, with higher scores indicating better motor function) - dif-

TABLE 2 - BENEFIT AND/OR HARM - AT 15 MONTHS

OUTCOME	INTERVENTION (N = 84) Minimum mean Square (95% CI)	COMPARISON (N = 42) Minimum mean Square (95% CI)	Difference (95% CI)	p
Baseline change in HFMSE score	4.0 (2.9 to 5.1)	-1.9 (-3.8 to 0.0)	5.9 (3.7 to 8.1)	< 0.0001
Baseline change in Rulm score	4.2 (3.4 to 5.0)	0.5 (-0.6 to 1.6)	3.7 (2.3 to 5.0)	< 0.0001

ference of minimum mean square points = 3.7 (2.3 to 5.0), p<0.0001). **(A)**

- There is no difference in the number of adverse events. **(A)**

DISCUSSION

Two phase III clinical trials were included in this guideline. The first trial (Finkel, R.S. Et al., 2017)[3] assessed the use of intrathecal (IT) nusinersen with a dose adjusted according to the estimated volume of cerebrospinal fluid by age (equivalent to a dose of 12 mg for a 2-year-old patient) given on days 1, 15, 29 and 64 and maintenance doses on days 183 and 302, in SMA type I patients compared to a sham treatment. There was a reduction in the risk of death or use of permanent ventilatory support (47% lower in the nusinersen group than in the control group). However, when results were separated for each type of outcome (death and permanent ventilatory support), the results indicated a statistically significant difference between the nusinersen group and the simulated procedure in overall survival (risk of death) with HR=0.37 and 95%CI 0.18 to 0 , 77, but not for permanent ventilatory support (HR=0.66, 95%CI 0.32 to 1.37). It is possible, however, that due to loss of data caused by the premature termination of the study, as well as a shorter duration of follow-up, the statistical power has been reduced. IT nusinersen proved to be safe, with no difference in the number of treatment-related adverse events between both groups.

A second clinical trial phase III (Mercuri, E. et al., 2018)[11], not included in the Canadian Agency for Drugs and Technologies in Health (CADTH) technology assessment because of the use of a treatment regimen or dose https://www.cadth.ca/sites/default/files/cdr/clinical/SR0525_Spinraza_CL_Report.pdf), assessed the use of IT nusinersen in patients with SMA type II.

In this study, the dose of IT nusinersen was 12 mg (in a solution of 5 mL), administered on days 1, 29, 85 and 274. There was an improvement in motor function (HFMSE score) from the start of the study in patients treated with nusinersen compared to control patients (minimum mean square difference, p<0.0001), but there was no difference between the percentage of children reaching at least one new WHO milestone, out of a total of six milestones.

Aiming at presenting health professionals with guidelines to enable them to provide the best care and the most advanced technologies, the UK government created The National Institute for Clinical Excellence (Nice) in 1999. To date, Nice has not published guidelines for the use of IT nusinersen in patients with 5q SMA. However, there is a scheduled date for publication (November 21, 2018; https://www.nice.org.uk/guidance/indevelopment/gid-ta10281).

In Brazil there are no therapeutic guidelines on the use of IT nusinersen for SMA 5q published at the moment (April 29, 2018) by the National Commission for the Incorporation of Technology in SUS (Conitec; http://conitec.gov.br/), although the drug is registered under Anvisa (http://portal.anvisa.gov.br/).

APPENDIX I

Clinical question

In children with spinal muscular atrophy (SMA) 5q, is the use of nusinersen effective and safe?

Eligibility criteria

The main reasons for exclusion were: they did not respond to the PICO and study design.

Only studies with a randomized controlled clinical trial (RCT) design were included.

Search for papers

Database

The scientific information databases consulted were Medline/PubMed, Central (Cochrane) and Lilacs via VHL.

Identification of descriptors

P	Spinal muscular atrophy
I	Nusinersen
C	Sham procedure or conventional therapy
O	Clinical outcomes

Research strategy

Medline/PubMed: (Spinal Muscular Atrophies of Childhood OR Muscular Atrophy, Spinal) AND (nusinersen OR Oligonucleotides, Antisense)

Central (Cochrane): (Spinal Muscular Atrophy OR Spinal Muscular Atrophy) AND nusinersen

Lilacs via VHL: (Spinal Muscular Atrophy OR Spinal Muscular Atrophy) AND nusinersen

Critical evaluation

Relevance - clinical importance

This guideline was prepared by means of a clinically relevant question in order to gather information in medicine to standardize approaches and assist in decision-making.

Reliability - Internal validity

The selection of the studies and the evaluation of the titles and abstracts obtained from the search strategy in the databases consulted were independently and blindly conducted, in total accordance with the inclusion and exclusion criteria. Finally, the studies with potential relevance were separated.

When the title and the summary were not enlightening, we sought for the full article.

Only studies with texts available in its entirety were considered for critical evaluation.

No restriction was made regarding the year of publication.

Languages: Portuguese, English, and Spanish.

Results application - External validity

The level of scientific evidence was classified by type of study, according to Oxford[12](Table 3).

TABLE 3 - RECOMMENDATION DEGREE AND EVIDENCE STRENGTH

A: Experimental or observational studies of higher consistency.
B: Experimental or observational studies of lower consistency.
C: Uncontrolled case/study reports.
D: Opinion deprived of critical evaluation, based on consensus, physiological studies or animal models.

The selected evidence was defined as a randomized controlled clinical trial (RCT) and submitted to an appropriate critical evaluation checklist (Table 4). The critical evaluation of RCT allows to classify it according to the Jadad score[13], considering Jadad trials <3 as inconsistent (grade B) and those with score ≥3 consistent (grade A).

TABLE 4 - GUIDE FOR CRITICAL EVALUATION OF RANDOMIZED CONTROLLED TRIALS

Study data Reference, study design, Jadad, level of evidence	**Sample size calculation** Estimated differences, power, significance level, total number of patients
Patient selection Inclusion and exclusion criteria	**Patients** Recruited, randomized, prognostic differences
Randomization Description and blinded allocation	**Patient follow-up** Time, losses, migration
Treatment protocol Intervention, control, and blinding	**Analysis** Intention to treat, analyzed intervention and control
Outcomes considered Primary, secondary, measurement instrument for the outcome of interest	**Results** Benefits or harmful effects in absolute data, benefits or harmful effects on average

During the critical evaluation, the Grade[15] (Grading of Recommendations Assessment, Development and Evaluation) discriminatory instrument was applied, using evidence of high and moderate quality. (Tables 5, 6 and 7)

The risks of bias identified in the studies selected were an early termination of the study due to benefits and different patients regarding previously known prognostic factors (common to both RCTs). The other parameters assessed for risk of bias were adequate in both RCTs (Tables 5, 6 and 7).

Method of extraction and result analysis

For results with available evidence, the population, intervention, outcomes, presence or absence of benefits and/or harmful events, and controversy must be specifically defined whenever possible.

The results will be presented preferably in ab-

TABLE 5 - RISK OF BIAS IN INCLUDED RCTS (GRADE[14])

Parameters evaluated	Finkel RS[3]	Mercuri E[11]
Adequate randomization?	Yes	Yes
Was the allocation blinded?	Yes	Yes
Were the patients analyzed in the groups for which they were randomized (was there IT analysis)?	Yes	Yes
Were the patients in the groups similar in previously known prognostic factors?	No	No
Was the study blinded?	Yes	Yes
Except for experimental intervention, were the groups treated equally?	Yes	Yes
Were the losses significant?	Early termination and ITT	Early termination and ITT
Was there an early termination of study due to benefits?	Yes	Yes
Did the study have an accurate estimate of the effects of the treatment?	Yes	Yes
Are the study patients similar to those of interest?	Yes	Yes
Are study outcomes clinically relevant?	Yes	Yes
Have potential conflicts of interest been declared?	Yes	Yes

ITT = intention-to-treat analysis

TABLE 6 - CRITICAL EVALUATION WITH THE GRADE[14] DISCRIMINATORY INSTRUMENT (FINKEL, R.S. ET AL., 2017[3] STUDY - SMA TYPE I)

Certainty assessment							Nº of patients		Effect	Certainty	Importance
Nº of studies	Design of the study	Risk of bias	Inconsistency	Indirect evidence	Imprecision	Other considerations	Intrathecal nusinersen	Sham	Absolute Risk (95% CI)		
Hine section 2 respondents (improved motor function) (follow-up: six months variation to; assessed with: Hammersmith Infant Neurological Examination - Hine section 2)											
Finkel RS[3]	randomized clinical trial	not serious[a,b]	not serious[c]	not serious	not serious	None	21/51 (41.2%)	0/27 (0.0%)	41.2% (27.7 - 54.7)	HIGH	CRITICAL

CI = confidence interval. Explanations. a. Early termination. b. Patients differ in previously known prognostic factors. c. not valuable

TABLE 7 - CRITICAL EVALUATION WITH THE GRADE[15] DISCRIMINATORY INSTRUMENT (MERCURI, E. ET AL., 2017[11] STUDY - SMA TYPE II)

Certainty assessment						
Study	Risk of bias	Inconsistency	Indirect evidence	Imprecision	Publication bias	Overall certainty of evidence
Mercuri E[11]	not serious [a,b]	serious[c]	not serious	not serious	None	MODERATE

Explanations: a. Early termination due to benefits . b. Patients with different prognostic factors at the beginning of the study, between the groups. c. There was an improvement of the motor function in the HFMSE and Rulm analyses with nusinersen. However, there was no difference in new WHO milestones.

solute data, absolute risk, number needed to treat (NNT) or number needed to harm (NNH) and, eventually, in mean and standard deviation values (Table 8).

TABLE 8 - WORKSHEET USED FOR DESCRIBING AND PRESENTING THE RESULTS FOR EACH STUDY

Evidence included
Study design
Selected population
Follow-up time
Outcomes considered
Expression of results: percentage, risk, odds, hazard ratio, mean

RESULTS

Studies returned (05/2018)

TABLE 9 - NUMBER OF PAPERS RETURNED FROM THE SEARCH METHODOLOGY USED IN EACH OF THE SCIENTIFIC DATABASES

DATABASE	NUMBER OF PAPERS
Primary	
PubMed-Medline	188
Central (Cochrane)	10
Lilacs via VHL	45

TABLE 10 - NUMBER OF PAPERS SELECTED

Type of publication	No. of papers	Included	Excluded
Randomized trial	2	2	0

Application of evidence - Recommendation

The recommendations will be elaborated by the authors of the review, with the initial characteristic of the synthesis of evidence, being subject to validation by all authors who participated in creating the guideline.

The available evidence will follow some principles of exposure: it will be by outcome and will have as components: number of patients, type of comparison, magnitude, and precision (standard deviation and 95% CI).

Its strength will be estimated (Oxford[12]/Grade[15]) as 1b and 1c (grade A) or strong, and as 2a, 2b and 2c (grade B) or moderate , weak, or very weak.

Conflict of interest

There is no conflict of interest related to this review that can be declared by any of the authors.

Final declaration

The Guidelines Project, an initiative of the Brazilian Medical Association in partnership with the Specialty Societies, aims to reconcile medical information in order to standardize approaches that can aid the physician's reasoning and decision-making process. The information contained in this project must be submitted to the evaluation and criticism of the physician responsible for the conduct to be followed, given the reality and clinical condition of each patient.

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Erysipelatoid Carcinoma

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SUMMARY

Erysipelatoid Carcinoma (EC), also known as Inflammatory Metastatic Carcinoma, is a rare form of cutaneous metastasis, secondary to an internal malignancy, more often related to breast cancer. Clinically, the lesion has a well-marked, bound erythematous appearance, much like an infectious process, such as erysipelas and cellulitis, these being the most common differential diagnoses. It is characterized by an acute or subacute appearance with an erythematous plaque, sometimes hot and painful, being more often situated in the primary tumor vicinity, especially in the thorax wall in the region of a mastectomy due to breast cancer. Here we present the case of a 75-year-old patient with ductal infiltrated carcinoma for 3 years, who presented an acute erythematous and infiltrated plaque in the region of a previous mastectomy, with a final diagnosis of EC.

KEYWORDS: *Breast neoplasms. Erysipeloid. Skin neoplasms. Inflammation/pathology.*

INTRODUCTION

Population aging due to increased life expectancy has led to a greater number of benign and malignant neoplasms cases and, consequently, their complications, including metastases.[1,2] These can be defined as a dynamic process where the primary tumor cells migrate to different sites, through some mechanisms, including hematogenous, lymphatic dissemination, direct implantation from surgical procedures and adjacent tissue invasion by contiguity.[3,4]

Cutaneous metastasis from malignant neoplasia does not constitute a common event when compared to the frequency of liver and lung metastatic sites. However, because macroscopic aspects can be present, there is a greater chance that it is diagnosed in the initial stages.[2-4]

Cutaneous metastases usually occur concurrently with metastases in the lungs, liver and lymph nodes, but metastatic dissemination may be the first warning, or even the first sign, to indicate an underlying malignant neoplasm presence.[4,5] In females, breast cancer was the most frequently associated with cutaneous metastasis, with up to 70% of total

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cases diagnosed with cutaneous metastasis. The dermatological clinical pattern of breast cancer metastasis is variable, predominantly metastases with a large number of non-painful papular-nodular, normochromic, or erythematous-violet clinical metastases, which are localized on the thoracic wall.[6-9]

Erisipelatoid Carcinoma (EC), also known as Inflammatory Metastatic Carcinoma, is a rare form of cutaneous metastasis secondary to internal malignancy. EC is an uncommon clinical finding, the incidence of cutaneous metastases to carcinomas varying from 0.7 to 10%.[10-12] Lesions may present more nonspecific characteristics, such as subcutaneous or intra-dermal nodules, single or multiple, of generally stony consistency, rapid growth, adhered, of varying coloration, that sometimes ulcerate. However, in a more characteristic way of the pathology, they may manifest as papillary erythematous skin lesions, infiltrated and with edema presence, well-demarcated and slightly endured, that resemble much to an acute infectious process, such as erysipelas and cellulite, these being some of your most common diagnoses.[13-16] EC is more often secondary to breast cancer and less common in stomach cancers.[17,18] It is marked by an acute or subacute appearance, with an erythematous plaque, sometimes hot and painful, being situated near the primary tumor, especially in the thorax wall in a mastectomy due to breast cancer.[19,20]

EC is subdivided into primary and secondary forms. In the primary form, both, carcinoma and inflammatory changes occur simultaneously in a previously normal breast. Whereas in the secondary form, inflammatory changes occur later in a breast with previous carcinoma.[9,12] EC spreads rapidly, both locally and systematically. The prognosis is adverse with less than 2.5 years of multimodality treatment. Axillary nodal involvement, younger age at diagnosis, African-American ethnicity and negative hormonal receptor status are some darker factors.[5,6,21]

Cutaneous metastasis denominate *en cuirasse* is the rarest cases, located exclusively on the chest wall and characterized by stiff, infiltrated sclerodermiform-like skin that attacks the scalp and is called neoplastic alopecia.[22,23] Immunohistochemical techniques, such as cytokeratin 7 and cytokeratin 20, contribute to elucidate the diagnosis. BRST-2 antigen and estrogen and progesterone receptors may be useful for the diagnosis.[3,5,9,15,16]

EC treatment previously consisted only of surgery. However, this produced a less than 10% 5-year survival rates. Mastectomy is currently preferred by conservative breast surgery, but only produces an overall survival of 12 to 32 months when used alone. Adjuvant radiotherapy has been shown to improve locoregional tumor control, but does not affect survival rates, so the multimodality therapy is now the preferred option.[12,19,21]

Chemotherapy, neoadjuvant and/or postoperative, can improve overall survival rates when combined with local modalities, such as surgery and/or radiotherapy. [10,12,13]

This paper aims to present the clinical picture of an unusual pathology with details of its clinical and histopathological characteristics so that it is considered as one of the differential diagnoses of skin disorders.

CASE REPORT

We present a patient, NSG, 75 years old, female, white, natural and resident of Rio de Janeiro, Brazil. She contacted the dermatology service complaining of skin inflammation. Her present disease history was characterized by the presence of eyelid-like papule-erythematous, edematous, erythematous cutaneous plaques with precise, slightly indurated borders that resembled an acute infectious process, such as erysipelas and cellulitis, in a mastectomy region, 20 days before. She denied having had a fever and had already had previous treatment with cephalexin without improvement.

At the examination, infiltrated and hardened erythematous plaques of inaccurate limits were found, occupying the thorax and right breast anterolateral region. (figure 1 and 2)

The previous pathological history consisted of ductal infiltrant carcinoma for 3 years in the right breast, treated with mastectomy and chemotherapy, Adriblastine and Genuxal, and radiotherapy.

Carcinoma Erysipelatoid, Angiosarcoma, and Erysipelas were then considered as diagnostic hypotheses.

To elucidate the case, complementary exams were conducted, such as a complete hemogram (Hemocytes: 4.12 / mm³, hemoglobin 11.9 mg / dl, hematocrit 36.5%, and leukogram with 6400 leukocytes (0-3 / 0-0-2-65 / 24-12)), and biochemistry, which did not present alterations.

An incisional biopsy was performed, showing, in small and medium magnification, a skin fragment in-

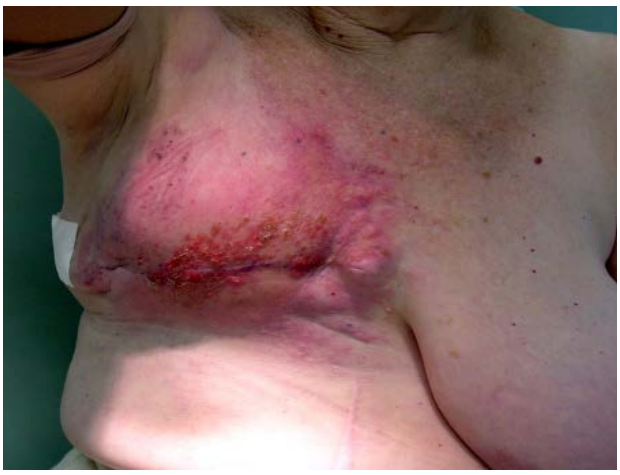

FIGURE 1. At the examination, infiltrated and hardened erythematous plaques of inaccurate limits were found, occupying the anterolateral region of the thorax and right breast.

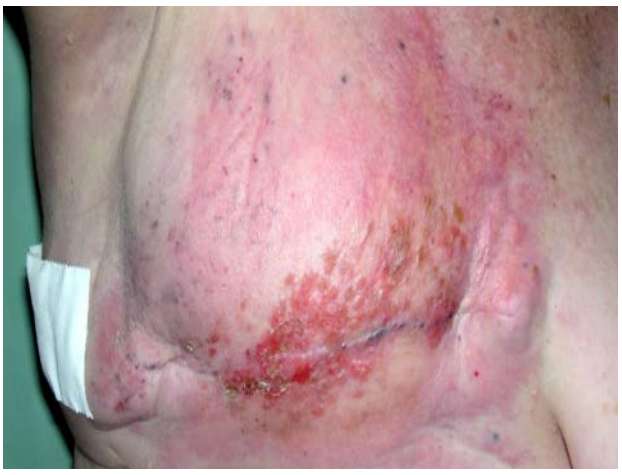

FIGURE 2. At the examination, infiltrated and hardened erythematous plaques of inaccurate limits were found, occupying the anterolateral region of the thorax and right breast

filtrated by adenocarcinoma with atypical neoplastic cells forming cords and groups of tumor cells in the reticular dermis. (figures 3 and 4). There was also a considerable increase of irregular aggregates of atypical epithelial cells forming strands and groups of cells that vary in size and shape, infiltrated between reticular dermis collagen fibers. (figure 5)

Immunohistochemistry was performed, revealing positive cytokeratin 7 and BRST-2 antigen (GCDFP-15) and negative cytokeratin20 and estrogen receptor. (figure 6 and 7)

In view of this, a diagnosis of Carcinoma Erisipelatoid diagnosis with mammary origin was established. After discussing the case with the oncology sector, chemotherapy was started with gemcitabine Hydrochloride and Paclitaxel. The patient presented partial edema and erythema regression after the 6th chemotherapy session.

It is noteworthy that the patient was previously and adequately informed about her pathological condition and its publication, consenting and signing the Free and Informed Consent Term.

DISCUSSION

EC is a rare form of cutaneous metastasis, secondary to internal malignancy and is commonly associated with breast carcinoma, especially with the intraductal carcinoma, presenting complications in 1-2% of malignant breast disease cases.[13,14,24] The cutaneous malignant neoplasm metastasis of primary internal organs is rare, but when they occur, the most common primary site is the breast, in an average of 25% of cases. It may also arise from other tumor sites, such as the pancreas, stomach, colon, rectum, prostate, lung, ovary, and melanoma.[2,3,5] Cutaneous metastases originating from breast cancer generally obey the principle of location according to a topographic region near the origin organ, so that the cutaneous metastasis that arises from breasts or lungs tend to be located in the thoracic area. Although the incidence of cutaneous metastases of all carcinomas is rare, it has been verified that it is mainly found among women with breast cancer.[3,4,16]

The present study patient presented, in her previous pathological history right, breast malignancy, namely ductal infiltrant carcinoma 3 years ago, when mastectomy and chemotherapy with Adriblastine and Genuxal were performed and combined with radiotherapy, which is in agreement with the literature. It is noteworthy that the original metastasis topography was maintained, since its site was at the right thoracic region and the intraductal infiltrant carcinoma was in the ipsilateral region of the tumor.

Erysipelatoid carcinoma is most commonly caused by mammary carcinoma but rarely associated with gastric adenocarcinoma, which shows that the present patient diagnosis is standard and truthful, once the epidemiology was maintained, because its site of origin was the ductal infiltrant carcinoma. [17,25,26]

In the present study, the patient presented in her disease history papuloerythematous, infiltrative-edematous, edematous cutaneous lesions with well-defined borders, slightly indurated, that resembled an acute infectious process, such as erysipelas and/or cellulitis.[13,14,16] This clinical aspect is

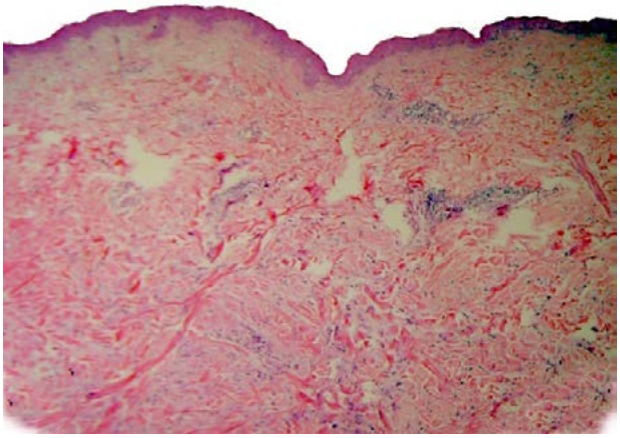

FIGURE 3. Photomicrograph showing fragments of skin infiltrated by adenocarcinoma with atypical neoplastic cells, forming cords and groups of tumor cells in the reticular dermis.

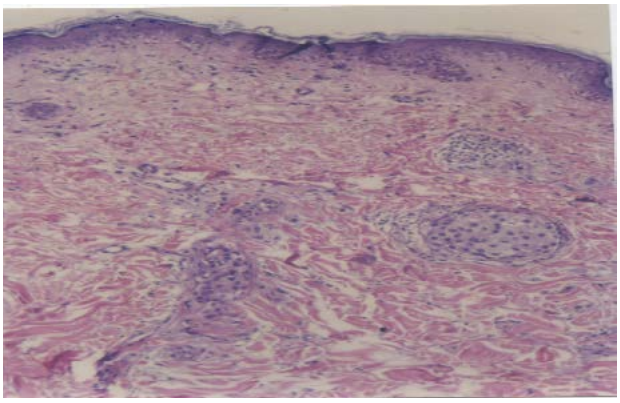

FIGURE 4. Photomicrograph showing fragments of skin infiltrated by adenocarcinoma with atypical neoplastic cells, forming cords and groups of tumor cells in the reticular dermis.

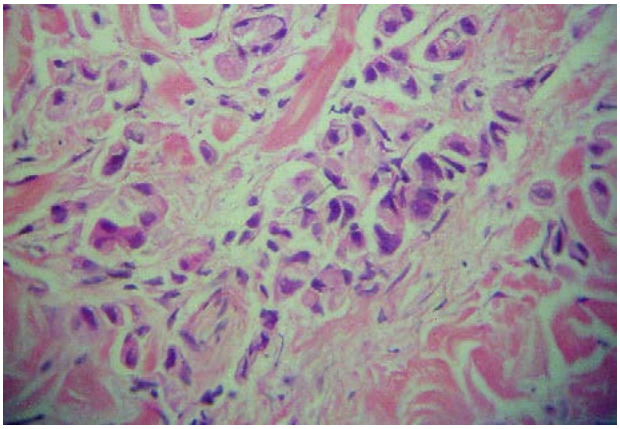

FIGURE 5. Presence of irregular aggregates of atypical epithelial cells forming cords and groups of cells that vary in size and shape infiltrated between collagen fibers in the reticular dermis.

characteristic of the pathology, since the literature describes this type of carcinoma as papular skin plaques with an erythematous aspect, infiltrated and with perilesional edema, well-demarcated and slightly indurated, so as to make a differential diagnosis from processes of infectious diseases such as cellulitis and erysipelas.

The skin lesion may persist for weeks or months, so that skin lesions often do not become apparent until some time has passed from the initial treatment of the original carcinoma. Its striking clinical aspect is difficult to diagnose and usually presents as a diagnostic challenge, since it can be easily confused with other clinical entities. It presents absence of fever or leukocytosis and negative bacterial culture, differentiating it from infectious processes such as erysipelas, cellulitis and mastitis.[9,12,13] Other differential diagnoses include congestion, thrombophlebitis, post-surgical lymphedema, allergic reactions, post-radiotherapy dermatitis, herpes zoster infection, and hematoma.[13,14]

Complaining of an erythematous lesion and edema presence in the mastectomy region for 20 days, the patient denied fever and had previously been treated with Cephalexin, without improvement. In addition, to elucidate the situation, a complete hemogram was obtained, revealing: red blood cells: 4.12 / mm³, hemoglobin 11.9 mg / dl, hematocrit 36.5%, and a leukogram with 6400 leukocytes (0-3 / 0-0- 2-65 / 24-12), and biochemistry that did not present alterations. Initially, an infectious process was considered, but the clinical context and history did not fully support this diagnosis. In general, specific clinical features, such as a lack of feverish response, the absence of leukocytosis and prolonged presence of lesions from weeks to months, along with an absence of reaction to antibiotics, should alert to the possibility of cutaneous metastasis. Such laboratory results and lack of improvement with treatment confirm the pathology of non-bacterial pattern, despite its particular clinical aspect.

Metastatic cutaneous lesions usually occur in the final stage of cancer indicating that it may already have spread. Most patients have synchronous metastases in other organs.[4,5,21] In the patient, however, only the breast was affected, and no other sites of metastatic implants were evident.

A cutaneous biopsy is usually necessary, revealing infiltration of tumor aggregates predominantly in dermal lymphatic vessels by neoplastic cells causing their obstruction. It should always be a differential diagnosis for the unilateral involvement of the thoracic wall erythematous aspect, which does not show improvement with antibiotic treatment and with an

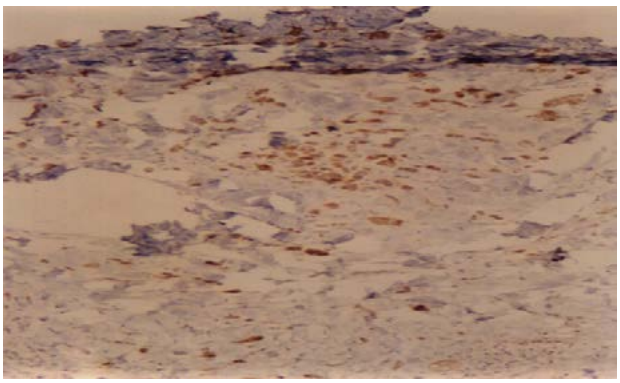

FIGURE 6. Ag BRST-2

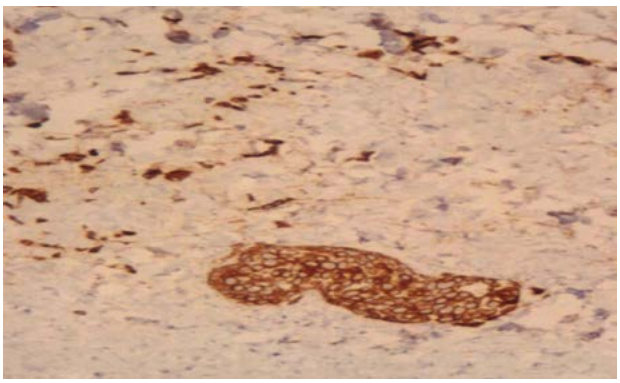

FIGURE 7. Citoqueratine 7

indurated appearance, mainly in patients with a previous history of malignancy. If the pathology is not correlated with the clinical findings, one should opt for deeper and repeated cutaneous biopsies if necessary.[13-15] Metastatic cutaneous inflammatory carcinoma has more than one clinical morphology, but the skin cells metastasis have metastatic tumor cells in common, predominantly located in dermal vessels, either lymphatic or blood vessels, and which may be present in the dermis between collagen bundles. Immunoperoxidase containing antibodies that bind to specific antigens, mainly in the lymphatic and/or blood vessels, allows the identification of dermal vessels infiltrated by the tumor.[4,5,18,19]

Immunohistochemical techniques, such as cytokeratin 7 and cytokeratin 20, contribute to elucidate the diagnosis. BRST-2 antigen and estrogen and progesterone receptors may also be useful for diagnosis[9,15-17,27] The patient presented immunohistochemistry, with positive cytokeratin 7, positive antigen BRST-2 (GCDFP-15), negative cytokeratin 20, negative estrogen receptor, evidencing and corroborating the disease pattern reflected by positive cytokeratin 7 and positive BRST-2 antigen.

The rapid clinical appearance and aggressive nature of EC require immediate diagnosis and therapy to be started as early as possible to improve patient survival.[13,17] Only with immediate recognition comes the opportunity to treat the systemic spread of cancer in the earlier stage as possible and improve survival rates. Thus, the response to induction chemotherapy is the most important prognostic factor.[16,17,19] The prognosis varies depending on the primary cancer type but, most of the time, it presents a limited survival rate.[5,11,28] That fact was decisive for beginning chemotherapeutic treatment in the patient.

EC treatment previously consisted only of surgery. Mastectomy is currently preferred by conservative breast surgery, but only produces an overall survival of months when used alone. Adjuvant radiotherapy has shown to improve locoregional tumor control but does not affect survival rates, and multimodality therapy is now the preferred option.[13,21,27,29] Chemotherapy with Gemcitabine Hydrochloride and Paclitaxel was initiated in the patient, with partial edema regression and erythema after the 6th chemotherapy session.

CONCLUSION

Cutaneous metastases are not common, but they are essential for signaling a possible primary cancer relapse or for alerting to possible occult neoplasia. Breast cancer one of the most common types of cancer that present skin metastasis and, when it does, it is usually to the chest wall. The dermatologic pattern of cutaneous metastases of breast cancer is that of solid, papulonodular lesions, but erysipeloid or armor infiltration is also possible.

After what was showed above, EC diagnosis should be considered in any persistent and therapeutically nonresponsive rash, like an infectious process, mainly in patients with previous malignant diseases.

Careful attention should be given to clinical features. Fever absence or leukocytosis such as persistence for a longer time, like weeks, contribute to a correct diagnosis of a noninfectious process.

Therefore, one should take a closer look at cutaneous lesions, especially those that affect patients with previous malignancies, so that diagnoses of less harmful malignancies are not considered as the first treatment option in patients with undiagnosed EC.

PALAVRAS CHAVE: *Neoplasias da mama. Erisipeloide. Neoplasias cutâneas. Inflamação/patologia.*

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Cutaneous lesions caused by the yellow fever vaccine – have you ever seen them?

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SUMMARY

The Yellow Fever virus was isolated in 1927 and the disease is considered endemic and epidemic in tropical regions of South America and Africa, with thousands of new cases reported annually. Several side effects of the vaccine have already been reported. Although reports of skin rash secondary to the vaccine range from 0 to 15%, no image or detailed description of the lesions were found in the literature. Here we describe a rash on a toddler vaccinated to travel.

KEYWORDS: *Exanthema. Drug eruptions. Adverse drug reaction reporting systems. Yellow fever vaccine.*

INTRODUCTION

The Yellow Fever virus (YF) was isolated in 1927[1] and is an endemic disease in tropical regions of South America and Africa, with thousands of new cases reported annually[2].

The causal agent of YF is an arbovirus and it is transmitted through the bite of an infected mosquito, the *Aedes Aegypti* in the urban areas[3].

Prior to the advent of the vaccine, YF was one of the most feared human infections due to hepatitis, renal failure, hemorrhage, shock, and death in 20-50% of cases[2].

Still in the 1930s, two vaccines were created, and during their development 32 laboratory workers contracted the disease and 15% of them died[1].

Adverse effects have been observed since 1930 in about 20% of cases[1]. Severe effects include anaphylactic reaction, neurological disease and viscerotropic disease[1] with an extensive list of contraindications to vaccination[4].

Although reports of rashes secondary to the vaccine can reach 15%, no images of such lesions were found in the literature.

CASE PRESENTATION

A healthy 18-month-old boy with no history of allergies received the YF vaccine isolated. After four days, erythematous papules appeared on palms,

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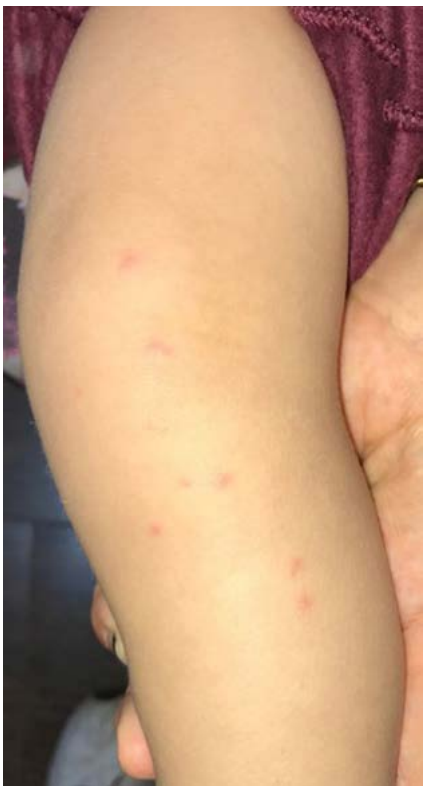

IMAGE 1. LESIONS ON THE LEG

IMAGE 2. ISOLATED ERYTHEMATOUS PAPULES

soles, trunk and limbs, including macular lesions on the conjunctiva (Image 1-2). There was no change in general condition, fever or pruritus. The lesions disappeared in three days without any treatment.

DISCUSSION

YF is an endemic disease in Brazil and the country has now the largest outbreak in the last decades, with a case fatality rate of 34.5% in 2017[5].

The Brazilian vaccine is an attenuated live virus preparation of the 17DD strain lineage provided by the Oswaldo Cruz Foundation - FIOCRUZ [6].

The reconstituted dose contains egg albumin, sucrose, sodium glutamate, sorbitol, bovine gelatin, erythromycin and kanamyci[7]. Cases of anaphylaxis may be secondary to the reaction to the egg protein or gelatin and have been reported in 1.8/100,000 doses[2]. However, a reaction to either component is possible, as well as to the latex from the vaccine vial lid[1].

Currently, it is questioned if vaccination should be limited to people traveling to areas where the risk of YF exceeds the risk of serious adverse events following vaccination[2].

A vaccine with inactive virus could reduce the risk of some of the adverse effects, especially of anaphylaxis, viscerotropic and neurotropic disease[2].

CONCLUSIONS

We report a case of a toddler with cutaneous reaction to the 17D YF vaccine showing the skin lesions of this adverse reaction.

RESUMO

O vírus da febre amarela foi isolado em 1927, e a doença é considerada endêmica e epidêmica em regiões tropicais da América do Sul e África, com milhares de novos casos relatados anualmente.

Vários efeitos colaterais da vacina já foram relatados. Embora os relatos de erupções cutâneas secundárias à vacina variem de 0% a 15%, nenhuma imagem ou descrição detalhada das lesões foi encontrada na literatura. Aqui descrevemos a erupção de uma criança vacinada para viajar.

PALAVRAS-CHAVE: *Exantema. Erupção por droga. Sistemas de notificação de reações adversas a medicamentos. Vacina contra febre amarela.*

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ORIGINAL ARTICLE

Repair of soft tissue defects of the fingers with medial plantar venous flap

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SUMMARY

OBJECTIVE: *To report the surgical procedures and efficacy of using medial plantar venous flap for the repair of soft tissue defects of the fingers.*

Methods: From March 2010 to April 2012, medial plantar venous flaps were harvested to repair the wounds of 31 fingers in 29 cases. Among them, there were 13 middle fingers with defects at the tips in 11 cases, 7 fingers with defects in the dorsal part in 7 cases, and 11 fingers with defects in the finger pulp in 11 cases. The size of the defects ranged from 1.2cm×1.5 cm to 2.5cm × 3.5cm. Medial plantar venous flaps of 1 .5cm × 2cm - 3×4 cm were harvested. Full-thickness skin grafts were adopted for the donor areas.

RESULTS: *All 31 flaps survived, except for 1 flap with arterial crisis and 2 cases with venous crisis. These conditions were timely corrected by secondary anastomosis of artery and vein and the flaps survived. The wounds and the donor areas achieved healing by the first intention. All grafted skins survived. Postoperative follow-up was conducted for 26 fingers in 24 cases for 4-12 months, excluding 5 cases with lost follow-up. The dorsal part of the damaged fingers had normal morphology, and the skin color and texture were similar to those of the normal skin. After the repair of defects in the fingertip and pulp, fingerprints appeared, and the protective sensation was restored.*

CONCLUSION: *The soft tissue defects of the fingers can be satisfactorily repaired with medial plantar venous flap, and little damage is caused to the donor area. This method is proven effective for the repair of soft tissue defects of the fingers.*

KEYWORDS: *Finger injuries. Finger/surgery. Surgical flaps. Reconstructive surgical procedures/methods.*

Soft-tissue defects of the fingers are very common in the clinical practice and can be associated with the exposure of deep tendons and bone tissues, which adds difficulty to the surgery. Nakayama et al.[1] (1981) were the first to successfully establish the model of the arterialized venous flap, after which the venous flap has found extensive applications. We repaired soft tissue defects in 31 fingers of 29 cases using the medial plantar venous flaps, from March 2010 to April 2012. The patients generally achieved satisfactory outcomes.

MATERIALS AND METHOD

1. Subjects

Thirty-one injured fingers in 29 cases were selected (20 males, 11 females, aged 19-49 years, with an average age of 27). Among them, there were 13 middle fingers with defects at the tips in 11 cases, 7 fingers with defects in the dorsal part in 7 cases, and 11 fingers with defects in the finger pulp in 11 cases. There were 1 thumb, 15 index fingers, 12 middle fingers and 3 ring fingers wounded. As to the reasons of injury, 16 were caused by mechanical crush, 6 were

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hot-crush injuries, 6 electric saw accidents and 1 infection after trauma of the dorsal part. The size of the defects varied from 1.2 cm×1.5 cm to 2.5 cm×3.5cm.

2. Procedures

1. Repair of finger palmar skin defects with medial plantar venous flap: Radical debridement was performed conventionally. The medial plantar venous flap was designed based on the conditions of the wound. The dorsalis pedis artery with a parallel-oblique proximal orientation was located. Depending on the specific needs, 1-2 superficial veins with the diameter comparable to the vessel to be anastomosed were chosen as the trunk veins of the flap. The flap was designed based on the anatomical relationship between the wound surface and the defective artery. The skin and the superficial fascia were cut open along the markers. The flap was deeply dissociated at the trunk veins. The flap only carried the skin and a thin layer of subcutaneous tissue with the veins. There was no need to expose the trunk vein when dissociating the flap. The length of the vessel to be anastomosed should be longer than that of the flap. The flap was then grafted to the palmar skin defect. The wound surface was repaired while performing reverse bridging of the trunk vein to the artery. The direction of venous blood flow in the flap should be parallel with that of the venous valve (see Fig. 1 and 2).

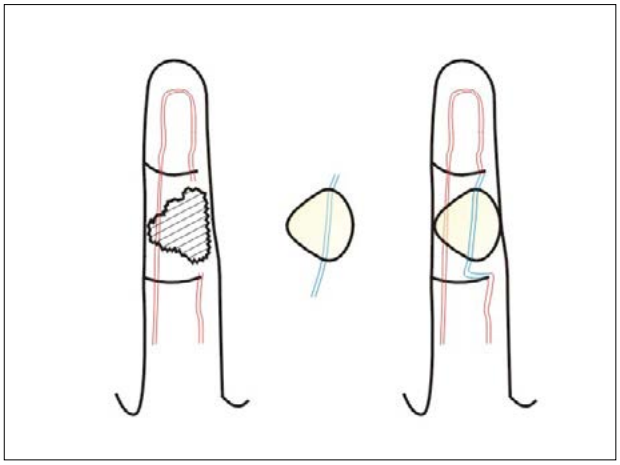


FIG. 1

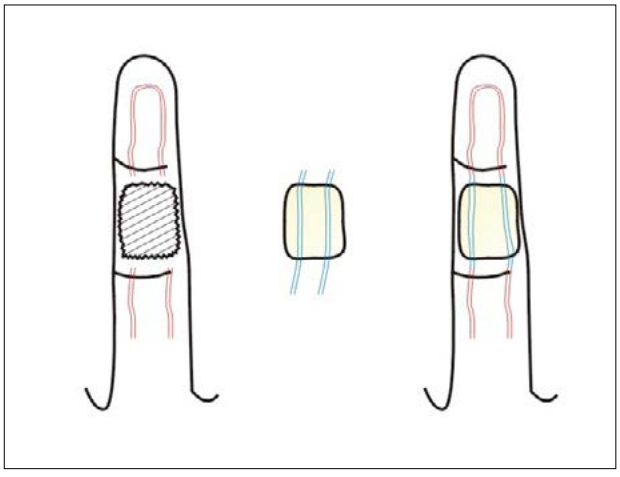


FIG. 2

Foundation: Scientific research program of Yiwu City (12-3-23)
Affiliation: Hand and foot surgery, Affiliated Yiwu Hospital of Zhejiang University.

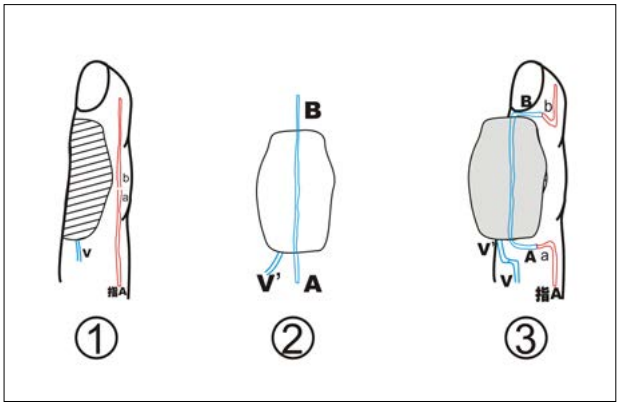


FIG. 3

2. Repair of defects in the dorsal part of the finger with medial plantar venous flap: Radical debridement was performed conventionally. The medial plantar venous flap was designed based on the conditions of the wound. The dorsalis pedis artery with a parallel-oblique proximal orientation was located. Depending on the specific needs, 1 superficial vein with the diameter comparable to the vessel to be anastomosed was chosen as the trunk vein of the flap. The flap was designed based on the anatomical relationship between the wound surface and the defective artery. The skin was cut open along the markers. The flap was deeply dissociated at the trunk vein. The flap only carried the skin and a thin layer of subcutaneous tissue with the vein. There was no need to expose the trunk vein when dissociating the flap. The length of the vessel to be anastomosed should be longer than that of the flap. One of two additional veins were preserved at the proximal end of the flap. The flap was then grafted to the wound surface in the dorsal part of the finger. The proper palmar digital arteries were dissociated and severed in the middle part of the wound surface. Anastomosis was performed between the proximal artery and the proximal trunk vein of the flap and between the distal artery and the distal trunk vein, so as to establish the proper palmar digital arteries. The spare vein of

the proximal flap was anastomosed to the proximal vein of the wound surface in order to ensure the venous reflux of the flap (see Fig. 3).

3. Repair of defects in the fingertip with medial plantar venous flap: Radical debridement was performed conventionally. The medial plantar venous flap was designed based on the conditions of the wound. The dorsalis pedis artery with a parallel-oblique proximal orientation was located. Depending on the specific needs, 1 superficial vein with the diameter comparable to the vessel to be anastomosed was chosen as the trunk vein of the flap. Alternatively, two veins showing an H-shaped orientation were chosen as the trunk veins of the flap. The flap was designed based on the anatomical relationship between the wound surface and the defective artery. When the defect was small, the venous flap was harvested from a single vessel; if the defect was large, two venous flaps with an H-shaped orientation were harvested. The skin was cut open along the markers. The flap was deeply dissociated at the trunk vein. The flap only carried the skin and a thin layer of subcutaneous tissue with the vein. There was no need to expose the trunk vein when dissociating the flap.

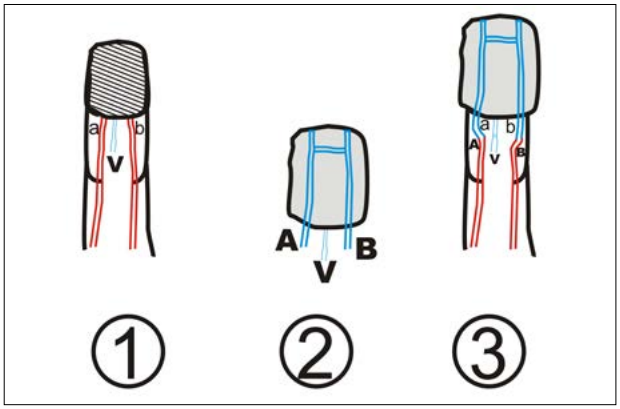


FIG. 4

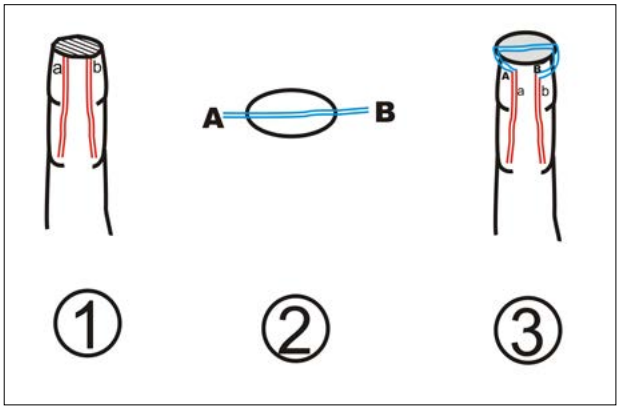


FIG. 5

The length of the vessel to be anastomosed should be longer than that of the flap. The flap was grafted to the wound surface. When a single venous flap was used to repair the wound surface, the proximal and distal ends of the veins were anastomosed to bilateral proper palmar digital arteries to form an arch-shaped loop of the arteries. When two venous flaps with an H-shaped orientation were used to repair the wound surface, the distal ends of the two veins were ligated, while the proximal ends were anastomosed to the proper palmar digital arteries. If the flap was large, one palmar digital vein was anastomosed to ensure the survival of the flap (see Fig. 4 and 5).

RESULTS

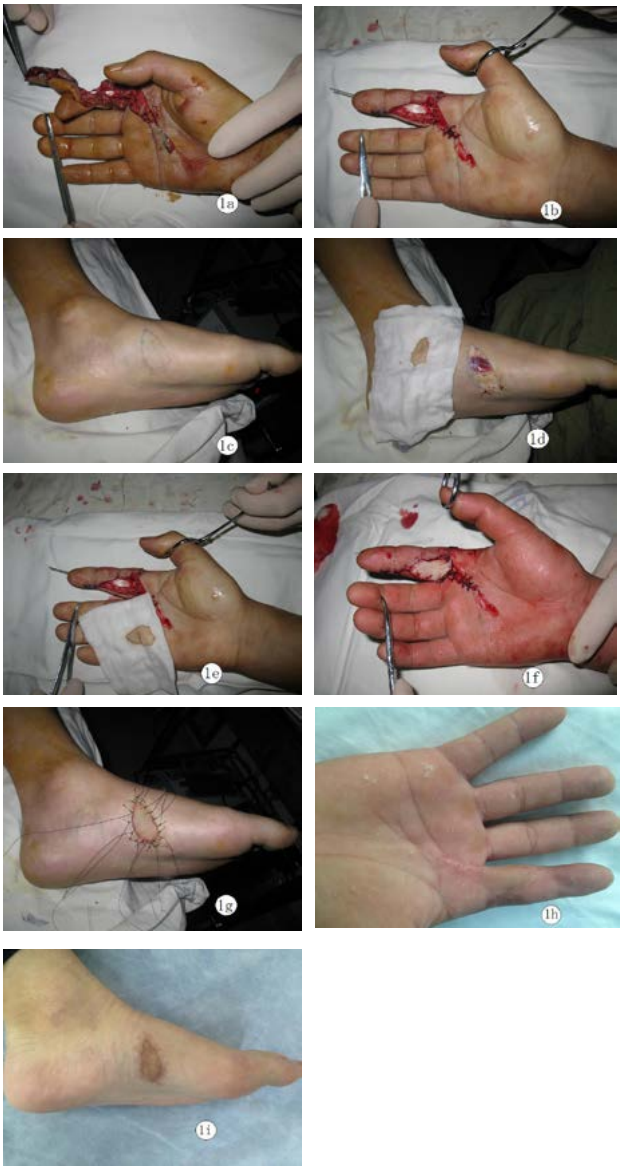
All 31 flaps survived. The flaps turned from light red to rosy 5-10 minutes after the blood was let through. The capillaries showed a normal response. The flaps were rosy 1-3 days after the operation, with normal response of capillaries but aggravated swelling. The flap color turned into dark red 4-7 days after surgery and the swelling persisted. The swelling receded later. The venous flaps were larger in size for 5 cases that had tension vesicles at 3 days after the operation, which disappeared spontaneously at 8-10 days. The other cases had no vesicles. One case had an arterial crisis and 2 cases had venous crisis. These cases received secondary anastomosis and embolism of the artery and vein, and all flaps survived. The wounds and the donor areas achieved healing by first intension, and the grafted skins survived. Twenty-four cases were followed up for 4-12 months, excluding those with lost follow-up. The dorsal part of the damaged fingers had normal morphology, and the skin color and texture were similar to those of the normal skin. After the repair of defects in the fingertip and pulp, fingerprints appeared, and the protective sensation was restored.

TYPICAL CASES

Case 1

The 30-year-old male patient was injured in the right index finger by mechanical crush, which led to the fracture of the proximal phalanx of the right index finger. There was a 1.5cm × 2.5cm soft tissue defect in the middle segment on the palmar side. The bilateral proper palmar digital arteries

ruptured. After emergency debridement, reduction fixation of the bone fracture was performed. A medial plantar venous flap of 2cm × 3cm was harvested. The two trunk veins of the flap were bridged to bilateral proper palmar digital arteries. A full-thickness skin graft was harvested to repair the skin defect. The flap survived, and the wound and donor area achieved healing by first intention. The skin graft survived. The color and texture of the flap were similar to those of the normal skin at the eighth month of follow-up. The patient was satisfied with the appearance of the operated site.

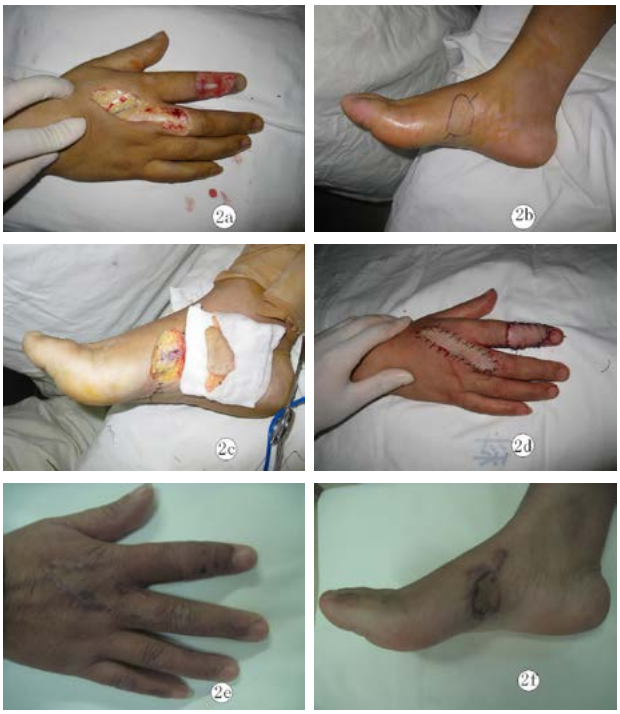


CASE 1. Fig. 1 a,b. Wound surface in the right index finger after the debridement; c. Design of flap from the donor area; d,e. Harvesting and transplantation of the flap; f. Flap immediately after the surgery; g. Skin graft from the donor area; h. Flap at 8 months after the surgery; i. Donor area at 8 months after the surgery.

The skin graft was normal, and no adverse impact was generated on the left foot mobility and weight bearing capacity.

Case 2

The 39-year-old female patient was injured in the dorsal part of the right index finger with third-degree scalding due to crushing by a stamping machine. After emergency debridement, a wound of 2.0cm ×4.0cm was found in the dorsal part of the right index finger, with partial damage to the extensor tendon. In addition, the extensor tendon and middle phalanx were exposed, with skin defects of 0.5cm × 1.0cm and 0.5cm × 0.6cm in the proximal and distal cross striations at the palmar side, respectively. In the dorsal part, there was a wound of 8.0cm × 2.0cm with moderately good subcutaneous tissues. The necrotic extensor tendon was removed, and a 2.5cm × 4.5cm medial plantar venous flap was harvested from the right foot. The free proper palmar digital arteries were harvested from the ulnar side and severed in the middle of the wound surface. Anastomosis was performed between the proximal and distal ends of arteries and the proximal and distal ends of



CASE 2. Fig. 2 a. Wound surface in the right index finger before surgery; b. Design of flap from the donor area; c. Immediately after the flap harvesting; d. Flap immediately after the surgery; e. Flap at 6 months after the surgery; f. Donor area at 6 months after the surgery.

trunk veins of the flap, respectively. The spare distal vein of the flap was anastomosed to the vein in the dorsal part. A full-thickness skin graft was harvested from the donor area. Other parts of the wound were repaired with an ilioinguinal full-thickness skin graft. The flap survived, and the wound and donor area achieved healing by first intention. During the 6-month follow-up, the color and texture of the flap were similar to those of the normal skin. The patient was satisfied with the appearance of the operated site. The skin graft was normal, and no adverse impact was generated on the right foot mobility and weight bearing capacity.

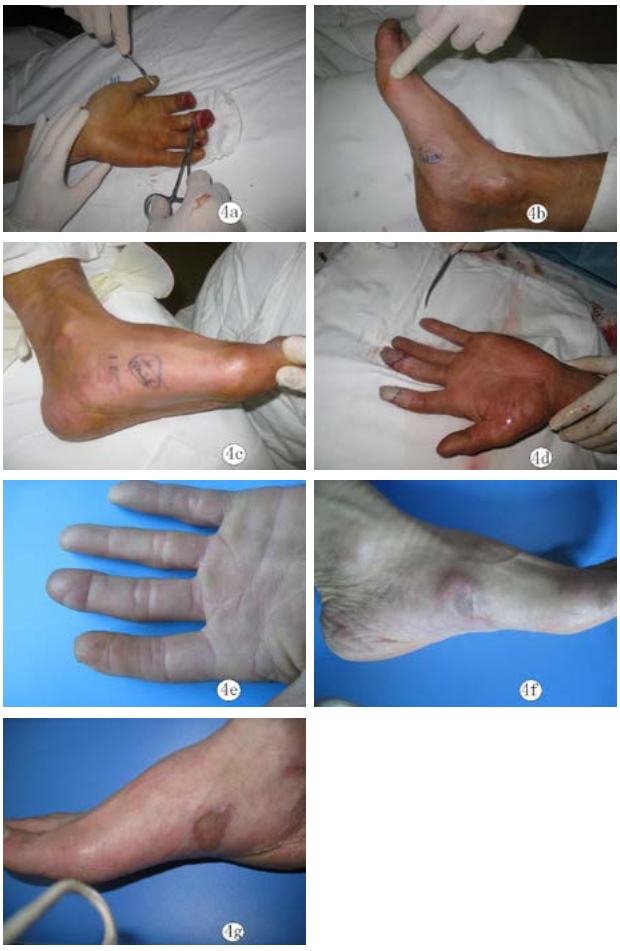
Case 3

The 43-year-old female patient lost the tips of the left thumb and left middle finger because of a mechanical crush. After radical debridement, an island-shaped flap was used to repair the defect in the left middle finger, and the medial tarsal flap for the left thumb. The defect in the left index finger was about 1.5cm × 1.5cm and was repaired with a 2cm × 2cm medial plantar venous flap from the right foot. The flap carried one trunk vein, and the proximal and distal ends of the vein were anastomosed to the bilateral proper palmar digital arteries, respectively. A full-thickness skin graft was harvested from the donor area. The flap survived, and the wound and donor area achieved healing by first intention. The skin graft survived. The color and texture of the flap were similar to those of the normal skin at 3 months after surgery. The patient was satisfied with the appearance of the operated site. The two-point discrimination of the flap was 10mm.

Case 4

The 45-year-old male patient lost the tips of the left index finger and middle finger due to mechanical crushing. The defect was about 1.8cm ×2.5 cm in the left index finger and 2cm × 3cm in the left middle

CASE 3. Fig. 3 a. Before the surgery; b. Design of the flap from the donor area; c,d. Flap harvesting and transplantation; e. Flap immediately after the surgery; f,g. Flap at 3 months after the surgery; i. Donor area at 3 months after the surgery



CASE 4. Fig. 4 a. Before the surgery; b,c. Design of the flap from the donor area; d. Flap immediately after the surgery; e. Flap at 6 months after the surgery; f,g. Donor area at 6 months after the surgery

finger. The distal phalanx was exposed with defects of the nail bed in the left middle finger. After radical debridement, a 2.5cm × 3cm medial plantar venous flap was harvested from the left foot to repair the defect in the left index finger, and a thin layer of nail bed from the right foot thumb for the left middle finger. The wound surface was repaired with a 2.5cm × 3.5cm medial plantar venous flap from the right foot. The two flaps carried two veins with H-shaped orientation, respectively. The distal ends of the two veins were ligated, while the proximal ends were anastomosed to the bilateral proper palmar digital arteries, respectively. One palmar digital vein was anastomosed. A full-thickness skin graft was harvested from the donor area. The flap and the transplanted nail bed survived. The wound and the donor area achieved healing by first intention. The skin graft survived. At 6 months after surgery, the color and texture of the flap were similar to those of the normal skin. The patient was satisfied with the appearance of the operated site. The two-point discrimination of the flap was 8mm.

DISCUSSION

1. Background

The repair of soft tissue defects of the fingers using miniature flaps has found extensive applications along with the development of the microsurgical technique. The flaps commonly used for the repair include free fibular skin flap of the foot thumb, tibial flap of the second toe, free flap based on superior-wrist cutaneous branch of the ulnar artery, interosseous dorsal artery flap and free perforator artery of the shank.[2-6] The size of the fibular skin flap of the foot thumb and tibial flap of the second toe should not be too large to prevent damage to the toes, and the flaps may sometimes die. Free flap based on a superior-wrist cutaneous branch of the ulnar artery and interosseous dorsal artery flap have the defect of a small diameter of the vessels and the donor area is less hidden, which affects the appearance of the forearm. The free perforator artery of the shank is larger in size, and the finger morphology and recovery of sensation may be poor after the repair. The above flaps are not fit for repairing the defects in bilateral proper palmar digital arteries and the wound surface simultaneously. In that case, the forearm venous flap is usually used.[7] Conventional venous flaps may have the defects of unstable blood supply, low survival, pigmentation and hard texture.[8,9] With the rising of people's living standard, the requirements on the repair aesthetics and functional recovery in hand injuries are also rising. There is also the need for harvesting flaps from hidden donor areas. According to Gu,[10] since the demand for repair aesthetics and functional recovery of hands is higher, it is better to harvest flaps from other positions, if possible. The medial plantar skin has a similar structure and texture as the palmar digital skin and therefore, it is fit for restoring the structure and morphology of the fingers. The fingerprints can be restored using the medial plantar skin, which is wear resistant, less slippery and conducive to hold objects. Therefore, the medial plantar region is an ideal donor area for the repair of soft tissue defects of fingers.

2. Survival mechanism of the arterialized venous flap (AVF): The survival mechanism of flaps is mainly related to blood circulation within the flap (balance between inflow and outflow). As to the survival AVF, Ji et al.[11] proposed 3 pathways for the AVF to acquire nutrients, in 1982: (1) The arterial blood enters the arterioles by the anastomosis between the venules and arterioles and then into the capillary network, where physiological perfusion takes place; (2) The arterial blood directly flows backward into the capillary network via the venules, where non-physiological perfusion takes place; (3) Blood circulation is formed between the flap and the normal surrounding tissues after some time; thus the blood is supplied to the flap. According to Imanishi et al.[12], communicating branches exist between the microvenous connection, accompanying veins and superficial venous network as well as between the microvenous connection, accompanying veins of the artery perforator, and paraneural nexus. Venous valves are usually found within the communicating branches. However, the venous valves are thin and there is the risk of reverse flow when the venous pressure increases. Chen et al.[13] proposed a microcirculation pattern important for the early survival of the flap: the blood flow circulates from the thin veins to the communicating branches and then back to the thin veins. In the later stages, the new blood vessels at the basal part of the receptor area and the invading ones at the flap margins provide stable blood supply to the flap. Xia et al.[14] found through experimentation that in the early stage of venous flap transplantation, the anastomotic branches between the artery and vein rarely open; no blood flows through the arterioles, or the ar-

terioles were obstructed. Under the microcirculation microscope, a large number of anastomotic branches between the veins open. Therefore, even in the presence of local clogging, collateral circulation can be established to bypass the venous flaps or thrombi and to supply blood to the distal flap. This is regarded as the survival mechanism of AVF, which can be briefly described as the circulation of venules ⟶ microveins ⟶ microveins ⟶ venules. Liu et al.[15] proposed the pathway of blood supply to AVF based on clinical trials: arterial blood ⟶ trunk vein ⟶ venules ⟶ microveins ⟶ capillary network (substance exchange) ⟶ microveins ⟶ venules ⟶ trunk vein ⟶ reflux. Therefore, the survival mechanism of AVF mainly relies on the extensive communicating branches between the microveins, venules and trunk vein, with an effective blood circulation to ensure flap survival.

3. Postoperative changes of flap color and causes of the swelling: In the early days after flap transplantation (1-7d), microcirculation is not yet formed between the flap and the receptor area. Since the blood is supplied to the flap by the arteries and the number of open microvenous circulations increases, the perfusion of the flap increases. That means more blood flows into the flap than out of it. As a consequence, the flap is dark-red colored and swollen. After 7d, the swelling gradually recedes with the establishment of local microcirculation, and the flap color changes back to normal. The flap survives, and the venous arterialization is finished. When the venous flap is small, there are fewer vascular networks in it and the perfusion of the flap is low. It is easier to establish a microcirculation between the flap and the wound surface to promote reflux. Therefore, smaller flaps are more likely to survive with a lower possibility of vesicle formation. But in larger flaps, there are more vascular networks and the perfusion of the flap is large. With lower blood reflux, vesicles are more likely to appear.

4. Advantages of the medial plantar venous flap: The survival of venous flaps depends on the perfusion of the capillaries[16], which in turn is related to the number of capillaries. According to Xia et al.[14], abundant venous networks are the histomorphological basis for the survival of AVF. Moretti et al. (1959) believed that the number of superficial dermal microvessels is proportional to the skin thickness. The palmar and plantar skins are thick and have a higher capillary density in the papillary layer. This feature can increase the flap survival. The medial plantar veins have a thicker wall than the forearm venous flap and therefore are easier to be arterialized. The medial plantar veins have several anastomotic branches, and the venous return of the flap is more satisfactory, thus increasing flap survival. Sun et al.[17] found through clinical practice that medial plantar venous flap had a better blood supply, a higher survival rate and better quality of soft tissues after the repair than the venous flaps harvested from other positions.

5. Strengths and shortcoming of the flaps: Plantar skin has similar structure and texture as the finger skin and therefore is applicable to the repair of soft tissue defects on the fingers. The plantar skin is wear resistant, less slippery and has quick sensation.[18] The medial plantar veins have constant anatomy and occur in large quantities; they can be harvested with high availability and flexibility. Moreover, their diameter is similar to that of the proper palmar digital arteries, which makes anastomosis easier. For the repair of a defect in the proper palmar digital arteries, bridging of the blood vessels can be performed to restore blood supply to the fingers. The donor site of the medial plantar venous flap is hidden and does not bear weight. Less damage is caused to this donor site. However, the area of the medial plantar venous flap should not be too large, and the surgeons are expected to be skillful in the anastomosis of the blood vessels.

6. Matters deserving attention during surgery: (1) The venous flaps tend to swell immediately after surgery and then shrivel in later stage, so they should be slightly larger than the wound surface; (2) The orientation of the trunk vein should be marked, and longer proximal and distal ends of the trunk vein should be preserved to facilitate the anastomosis with the blood vessels in the receptor site. The free length of the proper palmar digital arteries in the receptor site should be sufficiently long to facilitate the anastomosis with the blood vessels and to avoid their reversal. In this way, the trunk vein can be enveloped within the flap as much as possible to increase the perfusion of the flap; (3) When the flap is larger, the returning veins should be anastomosed to improve the reflux to the flap veins. This is because improving the venous reflux is very important for ensuring the early survival of the flaps;[19] (4) Postoperative placement of the drainage tube: Considering the obstructed venous reflux and postoperative exudation of the flap, a drainage tube can be inserted to reduce hematoma and infection and to facilitate tissue healing and blood supply reconstruction.

RESUMO

OBJETIVO: *Relatar os procedimentos cirúrgicos e a eficácia do uso de retalhos plantares mediais venosos para reparo de defeitos de tecidos moles dos dedos.*

METODOLOGIA: *De março de 2010 a abril de 2012, foram colhidos retalhos plantares mediais venosos para reparar ferimentos de 31 dedos em 29 casos. Entre eles, 13 dedos médios com defeitos nas pontas em 11 casos, 7 dedos com defeitos na parte dorsal em 7 casos e 11 dedos com defeitos na polpa digital em 11 casos. O tamanho dos defeitos variava de 1,2 cm × 1,5 cm a 2,5 cm × 3,5 cm. Foram colhidos retalhos plantares mediais venosos de 1,5 cm x 2 cm a 3 cm × 4 cm. Foram adotados enxertos de pele de espessura total na área doadora.*

RESULTADOS: *Todos os 31 retalhos sobreviveram, com exceção de 1 retalho com crise arterial e 2 casos com crise venosa. Esses problemas foram corrigidos a tempo com anastomoses secundárias das artérias e veias e os retalhos sobreviveram. Os ferimentos e áreas doadoras atingiram cicatrização por primeira intenção. Todos os enxertos de pele sobreviveram. Foi realizado acompanhamento pós-operatório em de 26 dedos em 24 casos por 4 a 12 meses, sendo que dos casos tratados 5 não tiveram acompanhamento. As partes dorsais dos dedos lesionados apresentaram morfologia normal, com cor e textura da pele muito similares a da pele normal. Após o reparo dos defeitos nas pontas e polpas digitais, impressões digitais apareceram e a sensação protetora foi restaurada.*

CONCLUSÃO: *Os defeitos de tecido mole dos dedos podem ser reparados de forma satisfatória com retalhos plantares mediais venosos, com poucos danos à área doadora. Este método mostrou se eficaz para o reparo de defeitos de tecido mole dos dedos.*

PALAVRAS-CHAVE: *Lesões nos dedos. Cirurgia/dedos. Retalhos cirúrgicos. Procedimentos/métodos cirúrgicos reconstrutivos.*

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ORIGINAL ARTICLE

Acute kidney injury and other factors associated with mortality in hiv-infected patients

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SUMMARY

OBJECTIVE: *HIV-related mortality is still high, especially in developed countries. The aim of this study is to investigate factors associated to death in HIV-infected patients.*

METHODS: *This is a cross-sectional study with all HIV adult patients admitted to a tertiary infectious diseases hospital in Fortaleza, Northeast Brazil, from January 2013 to December 2014. Patients were divided into two groups: survivors and non-survivors. Demographical, clinical and laboratory data were compared and a logistic regression was performed in order to investigate risk factors for death. P values ≤0.05 were considered statistically significant.*

RESULTS: *A total of 200 patients with mean age of 39 years were including in the study, 69.5% males. Fifteen patients (7.5%) died. Non-survivors presented a higher percentage of males (93.3 vs. 67.3%, p = 0.037). Non-survivors presented AKI (73.3 vs. 10.3%, p < 0.001), liver dysfunction (33.3 vs. 11.5, p = 0.031), dyspnea (73.3 vs. 33.0%, p = 0.002) and disorientation (33.3 vs. 12.4%, p = 0.025) more frequently. Non- survivors also had higher levels of urea (73.8 ± 52.7vs. 36.1 ± 29.1 mg/dL, p < 0.001), creatinine (1.98 ± 1.65 vs. 1.05 ± 1.07 mg/dL, p < 0.001), aspartate aminotransferase (130.8 vs. 84.8 U/L, p = 0.03), alanine aminotransferase (115.6 vs. 85.4 U/L, p = 0.045) and lactate dehydrogenase (LDH) (1208 vs. 608 U/L, p = 0.012), as well as lower levels of bicarbonate (18.0 ± 4.7 vs. 21.6 ± 4.6 mEq/L, p = 0.016) and PCO2 (27.8 ± 7.7 vs. 33.0 ± 9.3 mmHg, p = 0.05). In multivariate analysis, disorientation (p = 0.035, OR = 5.523, 95%CI = 1.130 - 26.998), dyspnoea (p = 0.046, OR = 4.064, 95%CI = 1.028 - 16.073), AKI (p < 0.001, OR = 18.045, 95%CI = 4.308 - 75.596) and disseminated histoplasmosis (p = 0.016, OR = 12.696, 95%CI = 1.618 - 99.646) and LDH > 1000 U/L (p = 0.038, OR = 4.854, 95%CI = 1.093 – 21.739) were risk factors for death.]CONCLUSION: AKI and disseminated histoplasmosis (DH) were the main risk factors for death in the studied population. Neurologic and respiratory impairment as well as higher levels of LDH also increased mortality in HIV-infected patients.*

KEYWORDS: *HIV. Mortality. Risk factors. Histoplasmosis. Lactate dehydrogenase.*

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INTRODUCTION

According to the United Nations Program on HIV/AIDS (UNAIDS)[1], an estimated 36.7 million people worldwide were living with HIV in 2015, while 2.1 million people were infected in the same year. This alarming epidemiologic situation confirms the role of HIV infection as an undoubtedly severe public health issue, which leads to an estimated annual spending of 19 billion dollars and over a million deaths every year[1].

In the late 90s, the association of multiple antiretroviral drugs was a milestone in the management of HIV infection, initiating the highly active antiretroviral therapy (HAART) era. The access to HAART has increased since then, reaching more than 17 million people in 2015[2]. Several studies have demonstrated the efficacy of such treatment in reducing viral RNA copies, preserving CD4 cells, and consequently decreasing morbidity and mortality[3]. On the other hand, HAART has been associated with significant adverse effects, such as liver dysfunction, skin rash, hypertension, diabetes, dyslipidemia, myocardial infarction and nephrotoxicity[4,5].

After the extensive introduction of HAART in developed countries of Western Europe and the United States in 1996, HIV-associated mortality has markedly decreased. In these countries, the leading causes of HIV-related death have been changing significantly, shifting from opportunistic infectious diseases to chronic conditions[6]. On the other hand, the national provision of HAART in Latin America started from 1997 to 2004 and Brazil was the first country in the region to establish policies to provide free and universal access to these medications. A study showed that only six out of eleven studied countries in the area presented a decline in HIV mortality from 1997 to 2007, probably due to this delay in implementing such free-access policies[7].

Considering the importance of the theme, the aim of this study is to investigate factors associated with death in HIV-infected patients.

METHODS

Study Design

This is a cross-sectional study with 200 HIV infected patients admitted to the São José Infectious Diseases Hospital, in Fortaleza, Northeast of Brazil, from January 2013 to December 2014. This hospital is a reference for all infectious diseases in the state of Ceará, in Northeast Brazil. All patients included were ≥ 16 years old, with a confirmed diagnosis of HIV infection. According to the protocol of the Brazilian Ministry of Health, at least two different tests must be used to confirm HIV infection, including enzyme-linked immunosorbent assay (ELISA), simple/rapid test devices, and western blot. All patients with previous estimated glomerular filtration rate (eGFR) <60ml/min/1.73m², heart failure, nephrolithiasis, use of nephrotoxic drugs (except for antiretrovirals), or other conditions that may affect renal function were excluded. Patients were divided into two different groups for comparison: those who died after admission (non-survivors) and those who did not (survivors).

Treatment

For those patients who were in HAART previously to admission, treatment included: zidovudine (AZT), didanosine (ddI), lamivudine (3TC), stavudine (D4T), abacavir (ABC), tenofovir disoproxil fumarate (TDF), lopinavir (LPV), saquinavir (SQV), ritonavir (RTV), amprenavir (APV), efavirenz (EFZ), and nevirapine (NPV), according to the protocols of the Brazilian Ministry of Health.

Definitions

Acute kidney injury (AKI) was defined and classified according to the Kidney Diseases Improving Global Outcomes (KDIGO) criteria[8]. Hypotension was defined as mean arterial blood pressure (MAP) <60mmHg, and therapy with vasopressors was initiated when the MAP remained <60mmHg, despite adequate fluid administration. Oliguria was defined as urine output <0.5ml/kg/h after adequate fluid replacement. Hemodialysis was indicated for those patients that remained oliguric after effective hydration, in cases with uremia-associated hemorrhagic phenomena or severe respiratory failure, as well as for patients with treatment-refractory hyperkalemia and metabolic acidosis.

Thrombocytopenia was defined as platelets count lower than 150,000/mm³ and anemia as hemoglobin (Hb) <12g/dL. The occurrence of metabolic acidosis was evidenced when pH < 7.35 and serum bicarbonate <20mEq/L, and severe metabolic acidosis when pH<7.10. Liver dysfunction was defined as the elevation of aspartate aminotransferase (AST) or alanine aminotransferase (ALT) >120U/L (three times higher than the normal range). All associated infections were diagnosed by the attendant clinicians during hospitalization and were confirmed by serologic or

laboratory tests, when possible. Disseminated histoplasmosis (DH), specifically, was diagnosed by identification of *H. capsulatum* in peripheral blood smear, bone marrow aspirate, blood and bone marrow culture, or histopathology of different tissues.

Studied parameters

Clinical and demographical parameters included age, gender, hospitalization time, main signs and symptoms on admission, the occurrence of associated infections, previous HAART use, AKI development, hemodialysis requirement, and death. All infectious diseases other than HIV presented by the patients, opportunistic or not, were considered "associated infections". Laboratory assessment included: mean HIV viral load, CD4 lymphocytes count, serum urea and creatinine, hemoglobin (Hb), hematocrit, leukocytes count, platelets count, sodium, potassium, aspartate amino transaminase (AST), alanine aminotransaminase (ALT), and lactate dehydrogenase (LDH). Arterial blood gas analysis included arterial pH, carbon dioxide partial pressure (pCO_2), and bicarbonate (HCO_3).

Statistics

Statistical analysis was executed using the SPSS software for Windows version 20.0 (IBM, USA) and its results were expressed through tables. The Kolmogorov-Smirnov test was used for numeric variables in order to assess variable distribution. Variables with normal distribution were expressed through mean ± standard deviation (SD). Variables with non-normal distribution were expressed through median values. A comparison of categorical variables was executed using Pearson's Chi-square, while numerical variables were compared using Student's T-test (for variables with normal distribution) or Mann-Whitney U test (for variables with non-normal distribution). P values ≤0.05 were considered statistically significant. In order to evaluate risk factors for death, a logistic regression model was used for categorical variables. All variables with statistical significance in univariate analysis were included in multivariate analysis. Adjusted odds ratios (ORs) and 95% confidence intervals (CIs) were calculated.

Ethics

This study was reviewed and approved by the Ethics Committee of São José Infectious Disease Hospital.

RESULTS

A total of 200 patients were included in the studied, 69.5% male. The mean age was 39 years, ranging from 16 to 74 years. Fifteen patients (7.5%) died. The percentage of males was significantly higher in non-survivors than in survivors (93.3 vs. 67.3%, p=0.037). Almost half of the patients (49%) used

TABLE 1 – COMPARISON OF DEMOGRAPHIC DATA, CLINICAL PARAMETERS, COMORBIDITIES, AND OUTCOMES BETWEEN SURVIVORS AND NON-SURVIVORS.

	Survivors (N = 185)	Non-survivors (N = 15)	p
Age (years)	37.7 ± 12.1	41.8 ± 13.9	0.214
Gender (%)			
Male	67.6%	93.3%	0.037
Female	32.4%	6.7%	
Hospitalization time (days)	18 ± 15	8 ± 6	0.005
Previous HAART (%)	50.3	33.3	0.207
Comorbidities			
Diabetes Mellitus (%)	3.2	13.3	0.055
HVB Coinfection (%)	0.0%	0.0%	-
HVC Coinfection (%)	0.0%	0.0%	-
Outcomes			
AKI (%)	10.3	73.3	< 0.001
Hemodialysis (%)	2.7	46.7	< 0.001

HVB - hepatitis virus B; HVC – hepatitis virus C. Chi-square test and Student's T-test were used. P values <0.05 were considered statistically significant.

HAART previously to the admission, but there was no significant difference between groups (50.3 vs. 33.3%, p=0.207). Non-survivors presented a significantly shorter hospital stay (8 ± 6 vs. 18 ± 15 days, p=0.005), developed more AKI (73.3 vs. 10.3%, p<0.001), had more severe forms of AKI (Stage 3 – 53.3 vs. 4.5%, p<0.001) and needed more hemodialysis (46.7 vs. 2.7%, p<0.001) than survivors, as presented in Table 1.

Non-survivors also manifested higher incidence of liver dysfunction (33.3 vs. 11.5, p=0.031), dyspnea (73.3 vs. 33.0%, p=0.002), diarrhea (60.0 vs. 33.0 %, p=0.035), disorientation (33.3 vs. 12.4%, p=0.025) and oliguria (20.0 vs. 1.6%, p<0.001), as summarized in Figure 1. In general, there was no significant difference between groups in associated infection incidence (86.7 vs. 68.1%, p=0.133), but non-survivors presented disseminated histoplasmosis (26.7 vs. 3.2%, p<0.001) and cryptococcosis (13.3 vs. 2.7%, p=0.031) more frequently than survivors, as summarized in Figure 2.

Regarding laboratory evaluation, non-survivors presented lower levels of hematocrit (28.0 ± 9.1 vs. 32.5 ± 7.2%, p=0.023), bicarbonate (18.0 ± 4.7 vs. 21.6 ± 4.6 mEq/L, p=0.016) and PCO2 (27.8 ± 7.7 vs. 33.0 ± 9.3 mmHg, p=0.05), as well as higher levels of serum urea (73.8 ± 52.7vs. 36.1 ± 29.1 mg/dL, p<0.001), creatinine (1.98 ± 1.65 vs. 1.05 ± 1.07 mg/dL, p<0.001), aspartate aminotransferase (130.8 vs. 84.8 U/L, p=0.03), alanine aminotransferase (115.6 vs. 85.4 U/L, p=0.045) and LDH (1208 vs. 608 U/L, p=0.012), as presented in Table 2.

In multivariate analysis, disorientation (p=0.035, OR=5.523, 95%CI=1.130-26.998), dyspnea (p=0.046, OR=4.064, 95%CI=1.028-16.073), AKI development (p<0.001, OR=18.045, 95%CI=4.308-75.596), disseminated histoplasmosis (p=0.016, OR=12.696, 95%CI=1.618-99.646) and LDH >1000U/L (p=0.038, OR=4.854, 95%CI=1.093–21.739) were risk factors for death in HIV infected patients, as evidenced in Table 3.

DISCUSSION

Despite a remarkable decrease in HIV-related mortality after the introduction of HAART, its rates remain notably high, especially in developing countries. The leading causes of death in these patients have progressively changed, shifting from an infectious and AIDS-related disease majority to chronic and non-AIDS conditions predominance, such as hepatic, pulmonary, and cardiovascular illnesses[6]. In this context, the present work represents one of the main studies to investigate risk factors for death among HIV-infected patients in our region.

When analyzing demographic data, we noticed that the studied population included a majority of males, as well as young and middle-aged people (<40 years = 58%). Older age has frequently been linked to elevated mortality among HIV patients, due to a higher incidence of comorbidities and lesser adherence to treatment. On the other hand, in a large epidemiolog-

FIGURE 1 - Comparison of clinical presentation on admission between survivors and non-survivors.

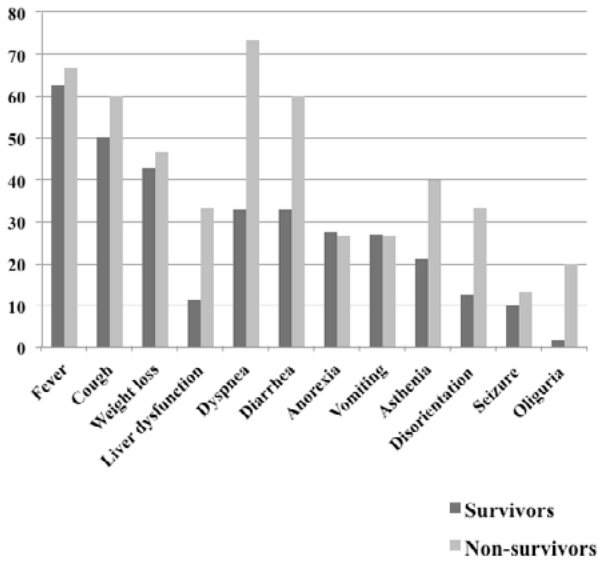
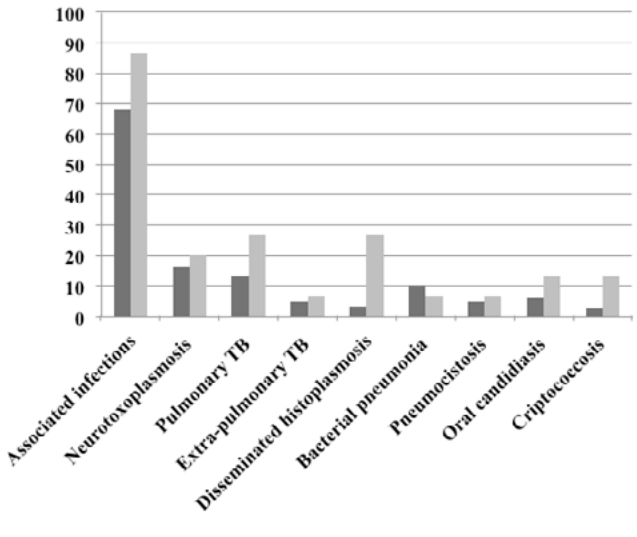


FIGURE 2 - Comparison of associated infections between survivors and non-survivors.



ical Chinese study, death was more common among those who were 20-49 years old, similarly to the majority of our patients[9].

In the present study, the percentage of males was higher among non-survivors than in survivors. Higher mortality in males has been extensively reported, mostly due to late referencing of men to HIV care and poor adherence to treatment[10]. Studies have demonstrated that women tend to initiate HAART sooner, which can be linked to HIV screening in prenatal exams for all pregnant women, which is mandatory in Brazil since 1996. On the other hand, men tend to look for medical attention only when they manifest symptoms, leading to late diagnosis and treatment[11]. We also observed that non-survivors had a shorter hospitalization time, mostly because they frequently died a few days after admission due to an acute condition or a complication.

Interestingly, HAART use was not significantly different between groups in the present study. Many studies have associated antiretroviral therapy with reduction of mortality, and its introduction may be considered one of the most important advances in the history of HIV infection, since it was responsible for reducing several complications and poor outcomes, including death[12]. Considering the fact that HAART use in the studied population did not influence CD4 lymphocytes count and viral load in both groups, we hypothesize that the lack of mortality decrease secondary to HAART use derives from a combination of irregular administration and insufficient information from charts. When data were collected, only basic information of previous HAART use was available in the charts (yes or no), but it was not described if these medications were being adequately taken by the patients, or how long they have been taking them. Hence, we believe that some patients who were classified as using HAART were not taking it correctly, leading to no difference between groups regarding HAART use, CD4 lymphocytes count, and viral load.

According to several studies, the general incidence of AKI on HIV-infected individuals is about 2.8-5.9% per year in outpatients, 6-18% in hospitalized patients, and 47.4% in critically ill patients[13-15]. In the present study, non-survivors had a significantly higher incidence of AKI and oliguria, needed more renal replacement therapy, and presented higher levels of urea and creatinine. Among hospitalized patients, AKI is more common in HIV-infected individuals than in the general population, as expected.

In the pre-HAART era, pre-renal azotemia was the unquestionable main form of AKI in HIV-infected patients, caused by volume depletion, associated infection, and sepsis[16]. On the other hand, in the post-HAART era AKI etiology changed and became multifactorial. Currently, volume depletion and sepsis are

TABLE 2 - COMPARISON OF LABORATORY DATA ON ADMISSION BETWEEN SURVIVORS AND NON-SURVIVORS.

	Survivors (N = 185)	Non-survivors (N = 15)	p
MVL (10^3 copies/mm^3)	187.3 (0 - 3707)	162.4 (0.3 - 639.2)	0.953
CD4 Lymphocytes (/mm^3)	277 (12 - 1456)	167 (38 - 363)	0.445
Hemoglobin (g/dL)	10.7 ± 2.5	9.5 ± 2.6	0.07
Hematocrit (%)	32.5 ± 7.2	28.0 ± 9.1	0.023
Leukocytes (10^3/mm^3)	6.88 (0.59 - 46)	12.42 (0.99 - 64)	0.323
Platelets (10^3/mm^3)	218.9 ± 107.7	166.0 ± 94.5	0.076
Urea (mg/dL)	36.1 ± 29.1	73.8 ± 52.7	< 0.001
Creatinine (mg/dL)	1.05 ± 1.07	1.98 ± 1.65	< 0.001
Sodium (mEq/L)	135.4 ± 5.2	133.0 ± 9.4	0.241
Potassium (mEq/L)	3.9 ± 0.7	4.8 ± 1.7	0.099
AST (U/L)	84.8 (7 - 1428)	130.8 (20 - 2180)	0.003
ALT (U/L)	85.4 (2 - 1316)	115.6 (8 - 118)	0.045
LDH (U/L)	608 (204 - 3294)	1208 (280 - 4184)	0.012
pH	7.40 (7.00 - 7.56)	7,35 (6.9 - 7.52)	0.674
HCO3 (mEq/L)	21.6 ± 4.6	18.0 ± 4.7	0.016
PCO2 (mmHg)	33.0 ± 9.3	27.8 ± 7.7	0.05

MVL – mean viral load; AST – aspartate aminotransferase; ALT – alanine aminotransferase; LDH – lactate dehydrogenase; HCO3 – arterial bicarbonate; PCO2 – arterial carbon dioxide partial pressure. Student's t-test and Mann-Whitney U test were used. P values <0.05 were considered statistically significant.

TABLE 3 – RISK FACTORS FOR DEATH AMONG HIV INFECTED PATIENTS.

Factor	OR	95%CI	p
AKI	18.045	4.308 - 75.596	<0.001
DH	12.696	1.618 - 99.646	0.016
Disorientation	5.523	1.130 - 26.998	0.035
LDH > 1000 U/L	4.854	1.093 - 21.739	0.038
Dyspnea	4.064	1.028 - 16.073	0.046

AKI – acute kidney injury; LDH – lactate dehydrogenase; DH - Disseminated Histoplasmosis. A logistic Regression model was used.

still important causes of AKI, but other factors like liver disease and drug toxicity have also become major etiologies[17]. Many conditions have been proposed as risk factors for AKI in this group, including low CD4+ count, elevated viral loads, previous renal disease, drug toxicity, underweight, older age, diabetes mellitus, cardiovascular disease, liver dysfunction, and hepatitis C coinfection[12,14]. Similarly to what we have observed in the present research, several other studies have demonstrated the role of AKI as a predictor of both short and long-term mortality in HIV-infected individuals, as well as its association with other poor outcomes, like heart failure and end-stage renal disease (ESRD)[14,18]. In the present study, severe forms of AKI were more prevalent in non-survivors. As previously described, they were considered an important risk factor for in-hospital HIV mortality[19,20].

Additionally, we noticed that patients in the non-survivors group had significantly lower levels of serum bicarbonate and arterial CO2 partial pressure, which represents a higher incidence of metabolic acidosis and hyperventilation. Acidosis has been recognized as a consequence of AKI in HIV patients and has been described as a risk factor for death in this population[21]. We believe that AKI was the primary cause of metabolic acidosis, but other factors may have contributed, such as drug toxicity by the nucleoside/nucleotide reverse transcriptase inhibitors (NRTI/NtRTIs)[22]. Several studies have demonstrated that drugs like stavudine, didanosine, zidovudine, lamivudine, abacavir, and tenofovir may cause acidosis through different mechanisms, such as mitochondrial dysfunction, Fanconi syndrome (proximal acidosis), and distal renal tubular acidosis[23,24]. In a previous study from our group with pediatric HIV patients, we observed that metabolic acidosis also happened as a consequence of HAART use[25].

Regarding liver involvement, non-survivors presented significantly higher levels of AST and ALT on admission. An extensive study by Weber et al.[26] showed that liver-related diseases caused 14.5% of all deaths and were the first cause of non-AIDS-related deaths. Many different factors may be linked to liver dysfunction in HIV patients, including hepatitis B (HBV) and C (HCV) coinfection, steatosis and non-alcoholic steatohepatitis (NASH), insulin resistance, drug toxicity, and direct effect of HIV[27]. It is difficult to define which factors are responsible for liver injury in our patients, but interestingly none of them tested positive for HBV or HCV. These infections share transmission routes with HIV and are observed in 5-25% of all HIV-infected patients worldwide but are usually overshadowed by opportunistic infections and hepatotoxic drugs as causes of liver injury[28].

Pulmonary infections are widespread in immunocompromised hosts, even in the HAART era and nearly 70% of HIV patients will experience respiratory complications at some point in their disease[29]. Pulmonary complications are the leading cause of hospitalization[30] and the leading causes of respiratory involvement in developed countries are bacterial pneumonia, bronchitis ,and *Pneumocystis jiroveci* pneumonia. Tuberculosis is still a frequent complication of HIV-infection in developing countries, while it is becoming rare in the developed world[31]. In the present study, dyspnea on admission was a risk factor for death in the multivariate analysis, which reflects pulmonary involvement. Other studies have shown that pulmonary infections are risk factors for death even in the HAART era, and HIV-patients are at higher risk of these conditions. However, it was not assessed whether the clinical symptom of dyspnea on admission was associated with higher mortality[32].

Additionally, disorientation was another important finding on admission. HIV-infection may cause disorientation for a myriad of reasons, including psychiatric comorbidities, immune dysfunction, HIV infection of the central nervous system (CNS), or progression of a systemic disease[33]. Although HAART treatment may prevent mental status alterations by precluding some of the above-mentioned complications, it can be the cause of the disorientation itself [34]. In the present study, disorientation was another risk factor for death, increasing mortality more than five-fold, in accordance with previous studies[35]. However, altered mental status has been demonstrated to increase mortality not only when there is ongoing HIV-associated CNS infection, but also in general wards[36,37]. Such findings suggest that the increase in

the risk of death derives primarily from the progression of the systemic disease, rather than from the infection of the CNS.

Among the myriad of HIV-associated infections, histoplasmosis is an important and dangerous global systemic mycosis caused by *H. capsulatum*. It is endemic in the Americas and has a notably high incidence in the state of Ceará, where this study was conducted[38,39]. It is considered an opportunistic infection which usually affects HIV patients with low CD4 levels, occurring in up to 5% of HIV infected patients in endemic areas[40]. Recent studies have stated that *Histoplasma* infection is more widespread than previously thought and its morbidity and mortality have been hugely underestimated due to severe underdiagnosing and underreporting[41]. The clinical presentation of this harmful disease may vary from asymptomatic infection with fever, chills, headache and malaise, to a life-threatening disseminated condition with septic shock, renal dysfunction, hepatic failure, acute respiratory distress syndrome and coagulopathy[39]. In addition, disseminated histoplasmosis [DH] has been described as an AIDS-defining condition, which usually occurs early in the course of HIV untreated infection and could even be the first manifestation of AIDS in some cases[42].

Unfortunately, poor outcomes are still very common in HIV patients with DH. Mortality rates among these patients can reach almost 50%, more prominently when severe manifestations like sepsis, renal failure, and pulmonary insufficiency are present[43]. In the studied population, DH was more common among non-survivors than survivors and it was a significant predictor of death in multivariate analysis. Histoplasmosis remains an important cause of death in HIV patients, and it has been described as the third most fatal mycosis among this population in Brazil[44]. Both early and long-term histoplasmosis mortality rates have been decreasing since the introduction of HAART, and antiretroviral use has been described as the most critical variable that influenced mortality reduction[45]. Several factors have been associated with mortality in patients with HIV-DH coinfection. Some authors demonstrated that high levels of creatinine and low levels of serum albumin were related to poor outcomes while others found that dyspnea, thrombocytopenia, and increased lactate dehydrogenase (LDH) levels were associated with mortality[40,46]. In a previous study from our group, other factors like diarrhea, neurologic manifestations, low hemoglobin levels, increases serum urea, liver involvement, respiratory insufficiency, and AKI were also linked to death in this population[47].

On the other hand, LDH levels were significantly lower in survivors group than in nonsurvivors, and LDH >1000U/L was a risk factor for death among our patients. In the present context, increased LDH levels are strongly related to histoplasmosis and may be used to differentiate this infection from *Pneumocystis jiroveci* pneumonia (PJP)[48]. In a previous study with HIV-infected patients from our group, we found higher levels of LDH in histoplasmosis than in other opportunistic infections[39]. Other authors have found that levels of LDH activity >2 times the upper limit of the normal range was a predictor of death[46].

In recent years, it has been stated that the significant LDH increase in histoplasmosis was associated with the development of hemophagocytic syndrome. This syndrome, also known as hemophagocytic lymphohistiocytosis (HLH), results from unregulated macrophage activation with overproduction of inflammatory cytokines, leading to hemophagocytosis[49]. It may be primary or secondary, triggered by hematologic malignancies, autoimmune diseases, or several infections, such as DH and HIV itself[50]. There is still scarce information about histoplasmosis-associated HLH, but it is recognized as a high-mortality condition[50]. Considering the intimate relationship between LDH and DH in the HIV setting, we believe that the role of elevated LDH levels as a risk factor for death derives from its connection to higher DH incidence and consequently more HLH. Since DH has been recognized as an underestimated and underdiagnosed global burden, further studies are necessary to investigate the connection between LDH, HLH, and mortality in DH patients.

In conclusion, neurologic impairment, pulmonary and respiratory involvement and AKI development were considered risk factors for death in HIV-infected patients. Disseminated histoplasmosis and elevated levels of lactate dehydrogenase (LDH) were also predictors of death in this population.

STUDY LIMITATIONS

This study has many limitations. The most important of them derives from its retrospective nature. Patients' data were retrospectively collect-

ed from medical charts, and we unfortunately did not have access to some of the patients' information. Also, retrospective studies are at higher risk of presenting biases. The significant difference in the number of patients in each group may be considered a limitation, since it makes statistical analysis more difficult and significantly influences results. However, we have chosen to compare these groups and investigate risk factors for death due to the importance of this subject, despite statistical difficulties.

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Conflicts of interest

We declare not having any conflicts of interest.

RESUMO

INTRODUÇÃO: *A mortalidade relacionada ao HIV ainda é alta, especialmente nos países em desenvolvimento. O objetivo deste estudo é investigar os fatores associados ao óbito em pacientes com HIV.*

MÉTODOS: *Trata-se de um estudo transversal com todos os pacientes com HIV admitidos consecutivamente em um hospital terciário de doenças infecciosas em Fortaleza, Nordeste do Brasil, entre janeiro de 2013 e dezembro de 2014. Os pacientes foram divididos em dois grupos: sobreviventes e não sobreviventes. Dados demográficos, clínicos e laboratoriais foram comparados e análise de regressão logística foi feita para investigação dos fatores de risco para óbito.*

RESULTADOS: *Um total de 200 pacientes, com média de idade de 39 anos, foi incluído no estudo, sendo 69,5% do sexo masculino. Óbito ocorreu em 15 pacientes (7,5%). Os não sobreviventes apresentaram maior percentual de homens (93,3 vs. 67,3%, p = 0,037) e um menor tempo de internação (8 ± 6 vs. 18 ± 15 dias, p = 0,005). Na análise multivariada, desorientação (p = 0,035, OR = 5,523), dispneia (p = 0,046, OR = 4,064), LRA (p < 0,001, OR = 18,045), histoplasmose disseminada (p = 0,016, OR = 12,696) e desidrogenase lática (LDH) > 1.000 U/L (p = 0,038, OR = 4,854) foram fatores de risco para óbito.*

CONCLUSÕES: *LRA e histoplasmose disseminada foram os principais fatores de risco para óbito na população estudada. Distúrbios neurológicos e respiratórios, bem como níveis elevados de LDH, também estiveram associados com o aumento da mortalidade em pacientes com HIV.*

PALAVRAS-CHAVE: *HIV. Mortalidade. Fatores de risco. Histoplasmose. Lesão renal aguda.*

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ORIGINAL ARTICLE

Percutaneous endoscopic debridement and irrigation for thoracic infections

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SUMMARY

OBJECTIVE: *To investigate the safety and efficacy of percutaneous endoscopic debridement and irrigation for thoracic infections and to make an appropriate choice according to the patient's condition.*

METHODS. *Thirty patients with thoracic infections who received surgical treatment from August 2014 to December 2016 were retrospectively analyzed. There were 16 males and 14 females, aged from 41 to 90 years, with an average of 64.4 years. A total of 9 cases were treated with percutaneous endoscopic debridement and irrigation (minimal group), and 21 cases were treated with open debridement in combination with pedicle screw fixation (conventional group). Patients underwent follow-up for 1 month. General condition, operative index, laboratory results, and imaging features were recorded.*

RESULTS. *Compared with the conventional group, there were more comorbidities in patients in the minimal group (8 cases in the minimal group, 10 cases in the conventional group, P=0.049), shorter hospital stay (10.1 + 2.26 days in the minimal group, 16.1 + 6.81 days in the conventional group, P=0.016), less bleeding volume (383.3 + 229.86ml in the minimal group, 90 + 11.18ml in the conventional group, P=0.000), lower VAS score at discharge (2.9 + 0.93 in the minimal group, 3.9 + 0.91 in the conventional group, P=0.013). There was no spinal instability case in the minimal group, 10 cases in the conventional group, P=0.013. There were significant differences. The C reaction protein prior to operation in the minimal group was 28.4±7.50mg/L. Compared with 45.1 + 15.78mg/L in the conventional group, P=0.005, it was lower.*

CONCLUSIONS. *Percutaneous endoscopic debridement and irrigation are an effective surgery for treatment of thoracic infections, especially suitable for patients with comorbidities and poor general condition. However, for severe infection and spinal instability, we tend to choose open surgery in combination with fixation.*

Keywords: Debridement. Endoscopy/methods. Infection.

With the aging of the population, the increase of underlying disease and immunosuppressed patients, and drug-resistant tuberculosis the incidence of spinal infections have increased[1]. Spinal infections include suppurative spondylitis, spinal tuberculosis, brucellosis, fungal infection, among others. It requires long treatment cycles and high costs, sometimes easily relapsing and even threatening patients' lives[2]. It is something that has become a heavy burden on families and society.

Infections, especially tuberculosis, easily affect the thoracic spine. It is different from the lumbar and cervical spine. Because of its adjacent position to the pleura and thoracic cavity and reduced blood supply for the spinal cord, the thoracic spine surgery is complex and high risk.

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The purpose of the operation is the identification of pathogenic bacteria species, debridement, correction of deformity, and reconstruction. Conventional surgery involves skin incision by posterior radical debridement, decompression, and pedicle screw fixation. This approach improves the rates of inflammatory relapse, but leads to surgical trauma, blood loss, and postoperative complication.

Minimally invasive surgery can avoid these disadvantages, it includes CT guided biopsy, minimal posterolateral decompression and fusion, percutaneous discectomy, tubular retractor system, among others. It can minimize injury to body tissues, reduce bleeding volume, and shorten postoperative bed rest[3]. Percutaneous endoscope for thoracic infection is rarely reported. Sometimes the thoracic spine is considered a restricted area for percutaneous endoscopic surgery. In our department, the percutaneous endoscope was used for thoracic infections, and the data was collected in order to analyze the effects of the surgery.

MATERIALS AND METHODS

Demographic Data of the Patients.

A total of 30 patients who underwent surgical treatment for thoracic infections in Qilu hospital and Jining No.1 people's hospital from August 2014 to December 2016 were retrospectively analyzed. The institutional review board of the Qilu Hospital and Jining No.1 People's Hospital approved this study, and all patients gave their informed consent. Of all patients, 16 were male, and 14 female. The age ranged from 41 to 90 years, with an average of 64.4 years. There were 7 cases of Pyogenic spondylitis, 21 cases of spinal tuberculosis, and 2 cases of brucellosis, all were thoracic infections. Patients were divided into two groups, percutaneous endoscopic debridement and irrigation group (minimal group) and open debridement and fixation group (conventional group). There were 9 cases in the minimal group, and 21 cases in the conventional group. General condition, operative index, laboratory results, and imaging features of the two groups were collected.

General condition included age, gender, smoking, comorbidity, and duration of hospital stay. Operative index consisted of bleeding volume, operation time, and preoperative, at discharge and 1-month postoperative VAS scores. The imaging features focused on abscesses, deformities, nerve deficit, and spinal instability. As for laboratory results, we chose C-reactive protein and erythrocyte sedimentation rate, which reflected the severity, progression, and therapeutic effect of the disease. Tumors and rheumatisms were excluded by laboratory tests and radiological imaging. Before surgery, all patients received antibiotics, but were not relieved.

Surgical Procedures.

Minimal group: Patients were prone positioned. The entry point was marked under fluoroscopic guidance. We used local anesthesia so that the patients were aware. Usually, the puncture point was 8-9 cm to the midline, parallel to the target intervertebral space, and the angle was 45 degrees from the coronal plane. The puncture needle was inserted into the disc through the foramen. The working cannula was placed and was connected with the Joimax endoscopy system. Aggressive debridement was carried out to remove nucleus pulposus, inflammatory granulations, and dead bones. Pathogenic specimens were sent for laboratory examination. Routine, smear, pathological, and etiological tests were performed. Mass antibiotic saline was irrigated, inflow and outflow. After thorough hemostasis, the drain tube was placed.

Conventional group: Before surgery, a biopsy was needed to identify pathogenic types. General anesthesia was adopted, and the patient was prone positioned. A midline incision was performed, and then through the paraspinal muscle approach, the articular process was exposed. Decompression was performed bilaterally through the articular process. The lamina was preserved without nerve compression. If there was nerve deficit, compression was needed to remove the lamina and open the vertebral canal. Nucleus pulposus, inflammatory granulations, and dead bones were cleaned up, and pathogenic specimens were sent for laboratory examination. Pedicle screws and titanium cage were inserted. After saline irrigation, two drain tubes were placed.

Postoperative management.

Appropriate antibiotics were selected according to a drug sensitivity test, isoniazid and streptomycin were selected for spinal tuberculosis, and tetracycline was chosen for brucellosis. The pathogen types couldn't be identified for 2 patients, but trial antituberculous therapy was effective, so they were considered to be atypical thoracic tuberculosis. Drainage

tubes were removed 7-10 days after surgery, and patients could practice early ambulation with the help of orthosis.

Statistical analysis.

All data was in the form of mean ±variance or cases-percentage. The sample size of both groups was less than 30 and did not coincide with a normal distribution; a nonparametric test was used. The Mann-Whitney test was used to compare independent measurement data, the Wilcoxon test was used to compare paired measurement data, and the Fisher exact test was used to compare enumeration data. P values <0.05 were considered statistically significant. Statistical analyses were performed using SPSS 21.0.

RESULTS.

There was no significant difference between the two groups in terms of age, gender, and smoking. Patients with comorbidities in the minimal group were 88.9%, higher than in the conventional group 47.6% (p<0.05). The mean hospital stay in the minimal group (10.1±2.26) was significantly shorter than in the conventional group (16.1±6.81). (Table 1 e 2)

There was no significant difference in operation time between both groups. The patients in the minimal group had less blood loss (383.3±229.86) than in the conventional group (90.0±11.18). There was no significant difference between the two groups of preoperative VAS scores. VAS scores in the minimal group at discharge were better than in the conventional group. But in the long term, there was no difference after the 1-month follow-up. (Fig. 1)

Among 9 patients who underwent minimally invasive surgery, 5 had abscesses, 1 had a deformity, 2 had nerve deficit, and there were no spinal instability cases. Among 21 patients who underwent conventional surgery, 11 had abscesses, 8 had deformities, 12 had nerve deficits, and 10 had spinal instability. Minimally invasive surgery and conventional surgery were both available for patients with abscesses, deformities, and nerve deficit; conventional surgery was not the only option. According to the Fisher exact test, there were significant differences between both cases of spinal instability. For patients with spinal instability, we chose to use pedicle screw fixation. (Fig 2)

The mean preoperative C-reaction protein in the minimal group (28.9±7.47) was lower than in the conventional group (45.1±15.78). There was no significant difference between discharge and 1-month postoperative. There was no significant difference in ESR between both groups either. ESR and C-reactive protein decreased gradually during the follow-up. (Table 3)

VAS scores, C-reactive protein, and erythrocyte sedimentation rate were selected as indications to evaluate the surgical effectiveness in the group that

TABLE 1. GENERAL CONDITION OF 30 PATIENTS WITH THORACIC INFECTIONS

	Minimal group (n=9)	Conventional group(n=21)	Statistical analysis	P value
Age(yrs)	66.7±9.51	63.4±12.22	Mann-Whitney test	0.504(p>0.05)
Female n (%)	5 (55.6)	9 (42.9)	Fisher exact test	0.694 (p>0.05)
Smoking n (%)	2 (22.2T)	7 (33.3)	Fisher exact test	0.681 (p>0.05)
Comorbidities n (%)	8 (88.9)	10 (47.6)	Fisher exact test	0.049 (p<0.05)
Hospital stay (days)	10.1±2.26	16.1±6.81	Mann-Whitney test	0.001 (p<0.05)

TABLE 2. OPERATIVE INDEX

	Minimal group (n=9)	Conventional group(n=21)	Statistical analysis	P value
Operation time (h)	2.6±0.63	2.9±0.68	Mann-Whitney test	0.811 (p>0.05)
Bleeding volume (ml)	383.3±229.86	90.0±11.18	Mann-Whitney test	0.000 (p<0.05)
VAS scores preoperative	5.0±1.80	5.4±1.08	Mann-Whitney test	0.594 (p>0.05)
VAS scores at discharge	2.9±0.93	3.9±0.91	Mann-Whitney test	0.028 (p<0.05)
VAS scores 1 month postoperative	1.6±0.53	1.4±1.03	Mann-Whitney test	0.504 (p>0.05)

TABLE 3. PREOPERATIVE AND 1-MONTH POSTOPERATIVE FOLLOW-UP VAS, CRP AND ESR

Case No.	VAS		CRP (mg/L)		ESR (mm/hr)	
	Preop	1-month Follow-Up	Preop	1-month Follow-Up	Preop	1-month Follow-Up
1	6	4	30.0	6.6	50	25
2	5	3	25.5	5.6	47	12
3	6	3	40.1	8.4	51	13
4	4	3	17.4	2.9	33	6
5	5	3	28.8	6.4	44	11
6	3	2	21.6	11.3	30	7
7	8	4	38.6	7.6	60	15
8	2	1	25.4	3.0	40	8
9	6	3	32.9	5.9	62	16
Mean ± SD	5.0±1.80	2.9±0.93	28.9±7.47	6.4±2.60	46.3±10.94	12.6±5.81
Wilcoxon test, P value	0.007(p<0.05)		0.008(p<0.05)		0.008(p<0.05)	

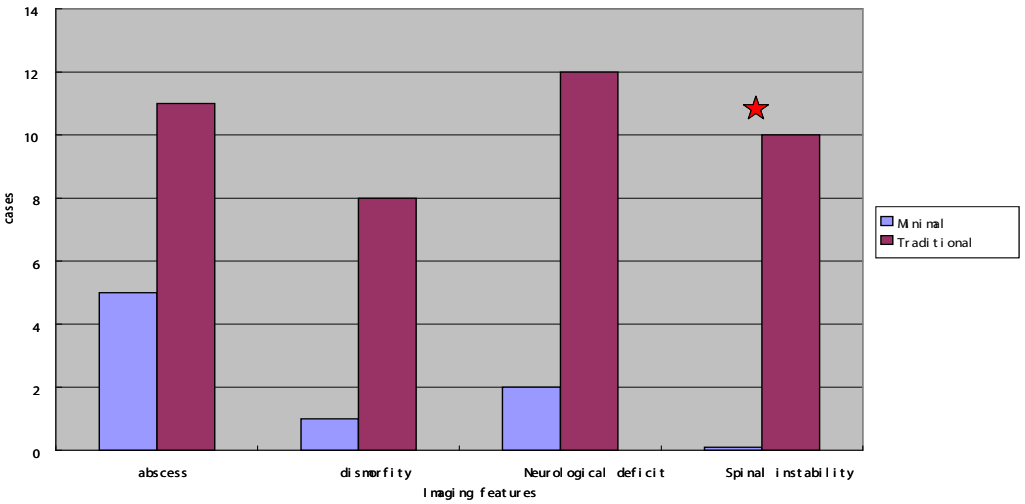

FIG.1. Comparison of imaging characteristics between the minimal and conventional group.

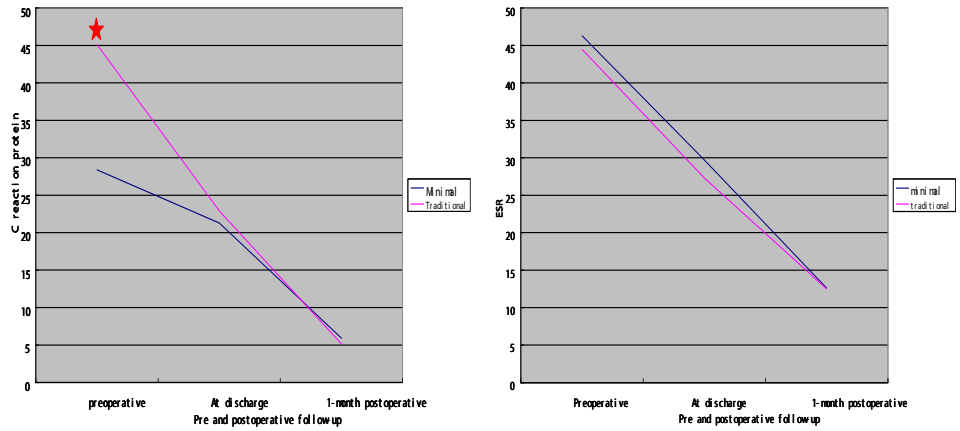

FIG.2. Preoperative, at discharge and 1-month postoperative follow-up C-reactive protein and erythrocyte sedimentation rate.

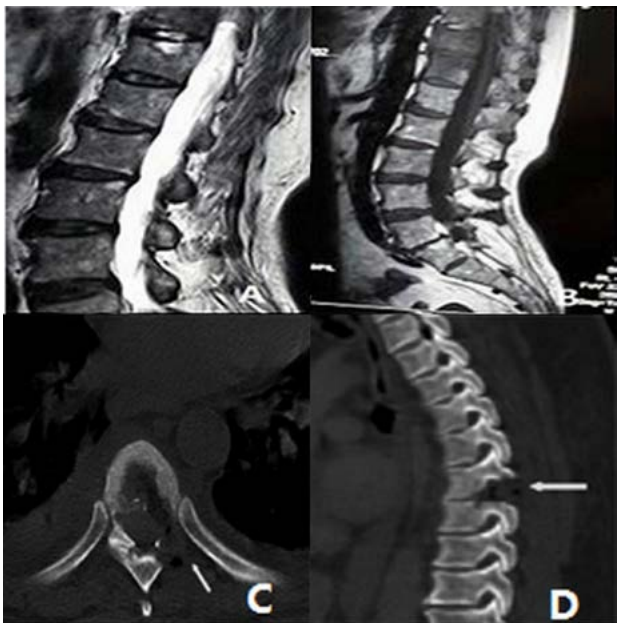

FIG.3. Preoperative and postoperative radiological images of patients.

underwent minimally invasive surgery. According to the Wilcoxon tests, there were significant differences between the preoperative and 1-month postoperative in three indexes. The VAS scores were lower, and inflammatory indexes were decreased; they were all improved. (Fig 3)

DISCUSSION

Conventional surgical treatments of thoracic infection are often accompanied by trauma and risk, which result in soft tissue injury, more bleeding, infection of peripheral structures, and slow recovery[4]. It is necessary to have a median incision from the posterior approach, the paraspinal muscle is stripped or injured, part of the lamina or articular process is resected, and the vertebral canal is decompressed. So many structures are affected that patients need long-term bed rest and rehabilitation exercises. The incidence of perioperative complications, such as thrombus, pneumonia, bedsore and infection is higher[5]. On the other hand, open surgery allows complete debridement, reconstruction, and stabilization of the thoracolumbar spine and is more suitable for patients with deformity and instability. Soft tissues are eroded by inflammatory substances and the local structure is poorly differentiated; a scar is formed in the operation area, and local anatomy is not very clear[6]. If treated improperly, it is easy to damage the surrounding structures. Some scholars are inclined to open surgery to avoid injury of important blood vessels and nerves. It is not suggested that open surgery is safer and minimal surgery is riskier. The choice of treatment depends on the technology available and the experience of the surgeons. Most people are not familiar with the minimally invasive approach of the thoracic spine, which causes the percutaneous endoscope not to be widely used.

Minimally invasive surgery has the advantages of minimal invasion, safety, and low cost, and it is easily accepted by patients[7]. Most patients want to solve the problem in one stage and prefer early ambulation. Open surgery sometimes requires preoperative biopsy and two stages: one for the posterior approach and another for the anterior approach[8]. The treatment process is long and complex for patients to endure. With the development of the minimally invasive technique, treating the patients safely and effectively has become a new direction[9]. However, we must be aware that it is not appropriate for everyone. It is better suited for patients with abscess and granulation, but it is not a good option for deformity and instability, so operation indications must be followed.

The percutaneous endoscope covers the shortage of CT guided biopsy and open surgery and is carried out to achieve the purpose of direct observation on lesions, taking out more pathogenic specimens, intervertebral disc and abscess cleaning, lavage and drainage[3]. It is safe and accurate, avoiding damage to spinal stability. Compared to CT guided biopsy, more pathogenic specimens are taken out to enhance the bioptic positive rate, in order to identify pathogen types. So, it simultaneously has two functions, examination and treatment. The surgery is accomplished in a single stage, so it is easily accepted by patients[10]. Conventional surgery usually requires preoperative biopsy and can be divided into more stages, anterior and posterior approach. The percutaneous endoscope is not as complicated, it is finished in one stage. For patients with severe underlying diseases, diabetes mellitus, coronary heart disease, and cerebrovascular diseases, and for patients who cannot tolerate major operations and need early mobilization, minimally invasive surgery is becoming the only option. It requires less time and causes less injury, with reduced effects on the heart, blood vessels, and lung and lower complication rates. The local anesthesia adopted can achieve quick recovery after surgery without the need to enter

the ICU[11]. The incidence of hypostatic pneumonia and thrombosis is obviously reduced.

There were few reports on the treatment of thoracic lesions by the transforaminal endoscope. It is a high risk that may cause damage to the spinal cord and pleura. The most critical step is the puncture. Affected by thoracic chest and ribs, the posterolateral approach is limited[12]. Compared with the lower lumbar spine, the puncture point is closer to the midline. The route is from 8-9cm laterally to the midline, through the intervertebral foramen, towards the vertebral disc. The angle should be more inclined to the ventral side, with 40 to 45 degrees to the coronal surface and parallel with the intervertebral space, so as to keep away from important structures, such as the thoracic cavity, rib, nerve root, and spinal cord[13]. It is conducted under X-ray guidance during its whole course. The thoracic spinal canal has little space reserved, and blood supply and tolerance of the thoracic spinal cord are poor. Any slight injury and disturbance may lead to irreversible injury of the spinal cord. Therefore, the movements should be slow and gentle to avoid sudden damage to the spinal cord. During this period, the activities of the lower extremities are closely observed.

Since infection frequently invades the anterior column of the vertebrae, the lamina and spinal canal are usually preserved, except for epidural abscess. Nerve deficit requires decompression of the spinal cord, including removal of the lamina and articular process, and complete removal of abscess and inflammatory granulation tissues. Epidural abscess is rare, but paravertebral and anterior vertebral abscesses are common. For patients with abscess, minimally invasive surgery has more advantages, because it can clean up necrotic substances and relieve pain symptoms.

Deformity and instability result from illness progression, and open surgery is required for correction and reconstruction. A pedicle screw was used extensively because of its strong intensity, so that the three columns can be firmly fixed[14]. The correction of kyphosis can be achieved by compression and distraction techniques. The technique of percutaneous endoscope cannot achieve the purpose of screw implantation and correction of deformities.

Before bacterial culture and drug sensitivity, antibiotics are empirically administrated. For gram-positive bacteria, especially methicillin-resistant Staphylococcus MRSA, vancomycin is more frequently used[15]. Cefoperazone or imipenem are chosen for gram-negative bacteria, tetracycline for Brucella, isoniazid and streptomycin for tuberculosis[16]. After the operation, appropriate antibiotics are selected according to the results of bacterial culture and drug sensitivity[17]. The application of antibiotics leads to a decrease of positive rate. Three days before the operation, it is suggested to stop antibiotics so as to improve the detection ratio of microorganisms[18]. Mycobacterium tuberculosis has a long culture cycle and low detection rate, and mostly relies on bacterial smears, acid-fast staining and pathology, Brucella relies on immunological examination[19]. Systemic administration may not achieve the aim of microbiological eradication, and irrigation with antibiotics effectively kills bacteria and prevents bacterial residue. Past opinions have suggested that local agents could produce drug-resistant strains, but that has not been observed. Irrigation can increase drug concentration in the lesions, especially for low concentration resistant pathogens.

Minimally invasive surgery still has risks of complications, paralysis caused by puncture injury of the nerve root and dural, hematoma caused by blood vessel injury, pneumothorax, intestinal fistula, and so on. Surrounding structure injured by puncture is an important cause of complications. Correct selection of surgical approach and avoiding puncture injury is the key to reduce complications. The range of vision under the endoscope is narrow, so surgeons should be familiar with microscopic anatomy and avoid blindfolded operation, hemostasis thoroughly and keep the drainage regularly so as to prevent hematoma and compression of spinal cord.

CONCLUSION

Percutaneous endoscopic debridement and irrigation for thoracic infection can reduce surgical trauma and shorten operation time and is especially suitable for patients with basic diseases and poor general conditions. It is an important supplement for the conventional treatment for thoracic infection, worthy of application.

RESUMO

OBJETIVOS: *Investigar a eficácia e segurança de desbridamento endoscópico percutâneo e irrigação torácica para infecções e fazer uma escolha adequada de acordo com a condição do paciente.*

MÉTODOS: *Trinta pacientes com infecção torácica que receberam tratamento cirúrgico de agosto de 2014 a dezembro de 2016 foram analisados retrospectivamente. Havia 16 homens e 14 mulheres, de 41 a 90 anos, com uma média de 64,4 anos. Nove casos foram tratados com desbridamento endoscópico percutâneo e irrigação (grupo mínimo) e 21 casos foram tratados com desbridamento aberto em combinação com fixação do parafuso pedicular (grupo convencional). Os pacientes foram submetidos a acompanhamento durante um mês. Estado geral, índice operacional, resultados de laboratório e imagem e funcionalidades foram gravados.*

RESULTADOS: *Em comparação com o grupo convencional, há mais comorbidades em pacientes do grupo mínimo (8 casos no grupo mínimo, 10 casos no grupo convencional, P = 0,049), menos tempo no hospital (10,1 + 2,26 dias no grupo mínimo, 16,1 + 6,81 dias no grupo convencional, P = 0,016), menos volume de sangramento (383,3 + 229,86 ml no grupo mínimo, 90 + 11,18 ml no grupo convencional, P = 0,000), menor pontuação no VAS a quitação (2,9 + 0,93 no grupo mínimo, 3,9 + 0,91 no grupo convencional, P = 0,013). Não houve nenhum caso de instabilidade espinhal no grupo mínimo, e 10 casos no grupo convencional, P = 0,013. Houve diferenças significativas. O nível de proteína C-reativa antes da operação no grupo mínimo era de 28,4±7,50mg/L. Em comparação com 45,1 + 15,78 mg/L no grupo convencional, P = 0,005, era mais baixa.*

CONCLUSÃO: *O método de desbridamento endoscópico percutâneo e irrigação é eficaz para o tratamento de infecções em cirurgia torácica, especialmente adequado para pacientes com comorbidades e mau estado geral. Mas, para a infecção grave e instabilidade vertebral, tendemos a escolher a cirurgia aberta em combinação com a fixação.*

PALAVRAS-CHAVE: *Desbridamento. Endoscopia/métodos. Infecção.*

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ORIGINAL ARTICLE

Ki-67 expression in mature B-cell neoplasms: a flow cytometry study

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SUMMARY

OBJECTIVE: *Ki-67 is a nuclear protein associated with cellular proliferation in normal or leukemic conditions that can help identify more aggressive diseases and is usually evaluated with immunohistochemistry. The aim of this was to assess Ki-67 expression on mature B-cell neoplasms samples with flow cytometry immunophenotyping.*

METHOD: *After surface staining with CD19 and CD45, intracellular staining for Ki-67 was performed in leukemic mature B-cells. Ki-67 expression was evaluated with flow cytometry.*

RESULTS: *Ki-67 expression was higher in mantle cell lymphoma, Burkitt lymphoma, and diffuse large B-cell lymphoma cases. It was also associated with CD38 mean fluorescence intensity.*

CONCLUSIONS: *Ki-67 expression evaluated by flow cytometry can be a useful tool in the diagnosis of mature B-cell neoplasms. More studies are needed to validate Ki-67 assessment with flow cytometry immunophenotyping.*

KEYWORDS: *Flow cytometry. Immunophenotyping. Ki-67 Antigen.*

INTRODUCTION

Mature B-cell neoplasms (MBCN) are a heterogeneous group of diseases have rearranged immunoglobulin gene, characterized by a monoclonal B-cell lymphoid population that usually has kappa or lambda restriction[1]. In the course of illness, there is a progressive accumulation of clonal cells, causing lymphocytosis, infiltration of bone marrow and other tissues[2]. The current classification of MBCN is based on the assessment of clinical, morphological, immunophenotypic, and genetic data [3].

Uncontrolled proliferation is a key feature of tumor cells and, in most cases, the percentage of proliferating cells provides the biological behavior and clinical course of the disease[4]. Ki-67 is a nuclear protein associated with cell proliferation that is expressed in all active stages of cell division, both in normal and leukemic cells, and contributes to the cell cycle regulation[5-7]. Therefore, Ki-67 is an excellent marker to establish the growth fraction of a cell population at a certain time, and the percentage of tumor cells expressing Ki-67 is used as a proliferation index for evaluating several types of cancer[8,9]. Most studies assessing Ki-67 expression in MBCN were performed with immunohistochemistry, which can be influenced by the presence of non-malignant reactive cells between tumor cells, different methodologies used in sample processing, and interoperator variations[10,11].

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This study was conducted to establish the expression of Ki-67 on B-cell lymphocytes of MBCN with a flow cytometry methodology.

METHOD

Flow cytometry staining for intracellular Ki-67 was performed in samples from 147 patients diagnosed with MBCN, according to the World Health Organization criteria[3]. The exclusion criteria were known previous treatment for B-cell neoplasms and presence of other hematological malignancies. Data were obtained in reports provided by the medical assistants.

The flow cytometric analysis was processed as reported elsewhere[12]. Briefly, samples were stained with fluorochrome-conjugated monoclonal antibodies (MoAbs) against CD45 FITC and CD19 APC. Red blood cells were lysed with Human BD Phosflow Lyse/Fix Buffer. Cells were washed and incubated with Fix/Perm Buffer (Transcription Factor Buffer Set). Cells were washed and incubated with Perm/Wash Buffer (Transcription Factor Buffer Set) and 5µL of Ki-67 PerCP-Cy 5.5 MoAb (clone B56). Cells were washed and resuspended in phosphate buffered saline (PBS). All samples were processed within 48-hours of collection[13]. All reagents were purchased from BD Biosciences (San Diego, CA, USA). Cells were acquired on a FACSCalibur flow cytometer using CellQuest software (BD Biosciences, San Diego, CA, USA). Analyses were carried out with CytoPaint Classic 1.1 (Leukobyte, Pleasanton, CA, USA). Expression intensities of CD19 and CD45 were used to gate B-cell population; the percentage and relative mean fluorescence intensity (MFI) of Ki-67 expression in $CD19^{+}/CD45^{bright}$ B-cell lymphocytes were recorded. MFI of CD5, CD10, CD19, CD20, CD23, CD38 and CD45 of MBCN cells were also registered.

Statistical analysis was performed with ANOVA and comparisons were adjusted by the Bonferroni test. The association of variables with Ki-67 MFI was evaluated by the correlation test of Spearman. Backward multiple linear regression, including variables associated with MFI of Ki-67 with $P<0.2$, was performed to identify factors independently associated with it. When variables had co-linearity, the more representative one was included in the analysis. Data were analyzed with SPSS v.18.0 (Chicago, IL, USA), and differences were considered significant when $P<0.05$.

This study was conducted from October 2014 to October 2015 in accordance with the Declaration of Helsinki and current laws in Brazil. This study was performed after approval of the Research Ethics Committee of the Hospital de Clínicas de Porto Alegre (14-0104). Written informed consent was deemed unnecessary.

RESULTS

This study included 94 chronic lymphocytic leukemias (CLL) (63.9%); 5 atypical chronic lymphocytic leukemias (aCLL) (3.4%); 19 marginal zone lymphomas (12.9%); 9 lymphoplasmacytic lymphomas

TABLE 1. CHARACTERISTICS OF THE SAMPLES INCLUDED IN THE STUDY, SEPARATED BY DISEASE.

	CLL (n=94)	aCLL (n=5)	LPL (n=9)	HCL (n=2)	MCL (n=9)	FL (n=5)	MZL (n=19)	BL (n=2)	DLBCL (n=2)
Sample									
Bone marrow	38 (40.4%)	1 (20.0%)	8 (88.9%)	2 (100%)	6 (66.7%)	4 (80.0%)	12 (63.2%)	1 (50.0%)	1 (50.0%)
Peripheral blood	56 (59.6%)	4 (80.0%)	1 (11.1%)	0	3 (33.3%)	0	7 (36.8%)	1 (50.0%)	1 (50.0%)
Lymph node	0	0	0	0	0	1 (20.0%)	0	0	0
Sample counts									
Lymphocytes (%)	69.53 (±18.23)	76.87 (±14.30)	39.10 (±24.82)	60.75 (±0.57)	58.73 (±26.19)	64.28 (±26.75)	45.68 (±21.40)	49.21 (±19.34)	62.55 (±24.02)
CD19+ (% of lymphocytes)	62.72 (±20.34)	66.78 (±19.51)	25.11 (±22.85)	20.46 (±17.09)	50.76 (±26.81)	51.60 (±23.80)	33.15 (±20.80)	38.30 (±29.95)	48.23 (±34.90)
MFI of CD20 on neoplastic cells	111.22 (±119.28)	89.16 (±31.73)	489.01 (±271.68)	787.59 (±354.94)	512.60 (±250.64)	397.76 (±211.61)	753.42 (± 484.89)	247.85 (±162.25)	203.13 (±105.90)
MFI of CD38 on neoplastic cells	7.75 (±8.00)	11.78 (±7.50)	22.42 (±20.46)	7.50 (±3.54)	30.48 (±26.71)	34.80 (±44.69)	9.86 (±8.05)	103.19 (±19.06)	40.46 (±49.17)

Data are shown as mean ±SD or number (n). Abbreviations: CLL: chronic lymphocytic leukemia; aCLL: atypical chronic lymphocytic leukemia; MZL: marginal zone lymphoma; LPL: lymphoplasmacytic lymphoma; MCL: mantle cell lymphomas; FL: follicular lymphoma; HCL: hairy cell leukemia; BL: Burkitt lymphoma; DLBCL: diffuse large B-cell lymphoma.

(6.1%); 9 mantle cell lymphomas (MCL) (6.1%); 5 follicular lymphomas (3.4%); 2 hairy cell leukemias (1.4%); 2 Burkitt lymphomas (1.4%) and 2 diffuse large B-cell lymphomas (DLBCL) (1.4%). Sample characteristics separated per disease category are shown in table 1. The expression of Ki-67 of MBCN samples included in the study is shown in table 2.

MCL cases had higher Ki-67 expression compared to CLL, aCLL, lymphoplasmacytic lymphoma, follicular lymphoma and marginal zone lymphoma cases ($P<0.05$). The MFI and percentage of Ki-67 expression were significantly higher in Burkitt lymphoma and DLBCL samples compared to the remaining cases, and Burkitt lymphoma cases had the highest Ki-67 MFI ($P<0.001$) (figure 1). Statistical analysis demonstrated the association of Ki-67 MFI in $CD19^+$ events with MFI of CD38 and CD20, and the percentage of CD19 and lymphocytes on the samples. After multivariable analysis, the MFI of CD38 expression remained significantly associated with the MFI of Ki-67 ($P<0.001$).

DISCUSSION

In our study, the MFI of Ki-67 among different MBCN was associated with the MFI of CD38 in leukemic cells. Also, the proliferation index – measured by the $Ki\text{-}67^+$ percentage and the Ki-67 MFI in $CD19^+$ events – was higher in MBCN that have more aggressive clinical courses compared to indolent diseases.

Few studies have evaluated Ki-67 expression with flow cytometry. Landberg and Roos[14] evaluated Ki-67 expression in non-Hodgkin lymphoma (NHL) cells and the percentage of expression allowed for the discrimination between high and low-grade lymphomas. In another study, Ki-67 staining was able to help differentiate between low, intermediate and high-grade NHL[15]. Our results are similar, with more aggressive diseases presenting higher proliferative index.

The Ki-67 expression in samples from CLL were evaluated with flow cytometry. Most studies evidenced low proliferative index, and higher Ki-67 expression was identified in samples from patients with advanced clinical stage or after cytokine stimulation[16-22]. Similar results were obtained with an in vitro proliferation study of MCL cells[23]. Those are in accordance with the low Ki-67 expression of CLL samples from our study, since we only included patients recently diagnosed. Given that our MCL group included some blastic variant samples, it had a higher and more variable Ki-67.

In a previous study, CLL subclones were separated according to their CD38 expression, Ki-67 expression was found with more frequency in the $CD38^+$ population and, cells with higher CD38 MFI presented higher Ki-67 percentage[24]. Lin et al.[25] sorted CLL cells from bone marrow or peripheral blood according to their Ki-67 expression; $Ki\text{-}67^+$ B-cells compartment contained a significantly higher number of $CD38^+$ leukemic cells compared to B-cells not expressing Ki-67. Interestingly, they did not find differences in Ki-67 or CD38 expression in samples collected on the same day from different sites of the same patient. CD38 is expressed by activated B-cells, and it was observed that CD38 expression was significantly higher in the proliferative fraction of CLL cells, this subset also had a higher number of cells with Ki-67 expression[26]. We found an association between the MFI of Ki-67 and CD38, considering all neoplasms and different sample types included in our study.

Herishanu et al.[27] used flow cytometry to evaluate the Ki-67 expression in CLL cells from different sites and identified higher expression on lymph nodes, compared to peripheral blood and bone marrow samples. We only had one lymph node sample from a follicular lymphoma case and its Ki-67 expression was low (5.5%, MFI of 10.2).

Immunophenotyping by flow cytometry is a method of cytological analysis that allows for the identification and characterization of cells in suspension[28,29]. A major advantage of flow cytometry

TABLE 2. KI-67 EXPRESSION ON MATURE B-CELL NEOPLASMS, SEPARATED BY DISEASE.

	CLL (n=94)	aCLL (n=5)	LPL (n=9)	HCL (n=2)	MCL (n=9)	FL (n=5)	MZL (n=19)	BL (n=2)	DLBCL (n=2)
Ki-67+ / CD19+ (% of total events)	1.63 (±1.32)	1.67 (±2.07)	0.81 (±0.70)	0.99 (±0.95)	11.99 (±16.72)	3.28 (±2.84)	1.56 (±1.25)	35.79 (±31.57)	38.10 (±30.17)
MFI of Ki-67 in CD19+ events	7.3 (±12.0)	4.6 (±1.7)	7.8 (±3.8)	8.8 (±1.3)	63.3 (±114.1)	21.3 (±32.9)	9.0 (±5.0)	501.6 (±490.8)	210.6 (±55.9)

Data are shown as mean ±SD. Abbreviations: CLL: chronic lymphocytic leukemia; aCLL: atypical chronic lymphocytic leukemia; MZL: marginal zone lymphoma; LPL: lymphoplasmacytic lymphoma; MCL: mantle cell lymphomas; FL: follicular lymphoma; HCL: hairy cell leukemia; BL: Burkitt lymphoma; DLBCL: diffuse large B-cell lymphoma.

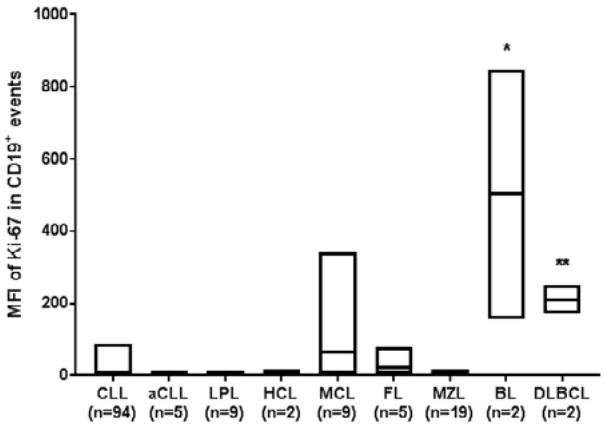

FIGURE 1 Box-plot diagram of Ki-67 mean fluorescence expression in mature B-cell neoplasms (the line shows the mean). Abbreviations: CLL: chronic lymphocytic leukemia; aCLL: atypical chronic lymphocytic leukemia; MZL: marginal zone lymphoma; LPL: lymphoplasmacytic lymphoma; MCL: mantle cell lymphomas; FL: follicular lymphoma; HCL: hairy cell leukemia; BL: Burkitt lymphoma; DLBCL: diffuse large B-cell lymphoma. * BL MFI was higher than all other groups, $P<0.001$. ** DLBCL MFI was higher than other groups, except BL cases, $P<0.001$.

is the possibility of methodology standardization for assessing multiple parameters on a single cell, as well as staining of more than one antigen[30]. The International Lunenburg Lymphoma Biomarker Consortium investigated the impact of immunohistochemical staining procedures and interoperator variation for the quantification of several markers in DLBCL and observed that Ki-67 expression had low reproducibility among the participant laboratories[11]. In our study, we used a flow cytometry approach to assess Ki-67 expression in different MBCN, flow cytometry has a methodological advantage compared to the current standard methodology for evaluation of Ki-67 expression, considering that immunophenotyping procedures can be standardized and validated for use in laboratories worldwide, the cell of interest can be gated, and test results are ready within hours.

Our study has some limitations. The major one is the inclusion of normal residual B-cells within the heterogeneous population of clonal lymphocytes on samples. Besides, MCL cases were considered together as one group, regardless of the variant type, and we had few cases of some types of MBCN. Also, we only had access to immunophenotyping and biopsy results as complementary tests for the disease entity definition, the latter provided by different laboratories.

CONCLUSION

In summary, in our study, Ki-67 expression was higher in MCL, Burkitt lymphoma and DLBCL cases and was associated with the MFI of CD38. The assessment of Ki-67 expression with flow cytometry has the potential to be used in the differential diagnosis of MBCN and other neoplasms. More studies are needed to compare Ki-67 expression with flow cytometry and immunohistochemistry in order to validate the flow cytometry methodology for diagnostic and research purposes.

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RESUMO

OBJETIVO: *Ki-67 é uma proteína nuclear associada à proliferação celular em condições normais ou leucêmicas que pode ajudar a identificar doenças mais agressivas. Este marcador é geralmente avaliado com imuno-histoquímica. O objetivo deste estudo foi avaliar a expressão de Ki-67 em amostras de neoplasias de células B maduras com imunofenotipagem por citometria de fluxo.*

MÉTODO: *Após marcação de superfície com CD19 e CD45, foi realizada marcação intracelular para Ki-67 em células B maduras leucêmicas. A expressão de Ki-67 foi avaliada por citometria de fluxo.*

RESULTADOS: *A expressão de Ki-67 foi maior em células de linfomas de manto, linfoma de Burkitt e linfoma difuso de grandes células B. Também houve associação de Ki-67 à intensidade de fluorescência média de CD38.*

CONCLUSÃO: *A expressão de Ki-67 avaliada por citometria de fluxo pode ser útil no diagnóstico de neoplasias de células B maduras. São necessários mais estudos para validar a avaliação de Ki-67 com imunofenotipagem por citometria de fluxo.*

PALAVRAS-CHAVE: *Citometria de fluxo. Imunofenotipagem. Antígeno Ki-67.*

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ORIGINAL ARTICLE

Evaluation of body image, quality of life, tactile sensitivity and pain in women with breast cancer submitted to surgical intervention

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SUMMARY

Breast cancer is one of the most common types of tumor in the world and the most common among women. There are several treatments for breast cancer; however, the condition often can be accompanied by severe complications in a woman's life.

OBJECTIVE: *o evaluate and compare body image perception, quality of life, tenderness, and pain in women with breast cancer during preoperative and postoperative periods of 30, 60 and 90 days.*

MATERIALS AND METHODS: *We conducted a prospective longitudinal study. The patients answered the questionnaire "How I relate to my own body", EORTC QLQ-C30 and EORTC QLQ-BR23. We assessed upper limb and breast sensitivity with an esthesiometer. Patients were questioned about the presence and level of pain on a scale of 0 to 10.*

RESULTS: *For body image, it was possible to observe a significant difference between pre and postoperative at 30 days. There were changes in some areas of the EORTC QLQ C30 and EORTC QLQ BR23 questionnaires, such as arm and breast symptoms, social function, constipation, sexual function and satisfaction, among others. For evaluation of breast and axilla sensitivity and assessment of pain, all postoperative periods showed significant differences when compared to the preoperative period. The sensitivity of the inner region of the arm presented no significant change.*

CONCLUSION: *The difference found in the study shows that evaluations on all scales should be done in several periods, using a proper treatment for the changes and individuality of each patient.*

KEYWORDS: *Signs and symptoms. Physical therapy modalities. Body image. Breast neoplasms.*

INTRODUCTION

According to the Ministry of Health, breast cancer is the leading cause of cancer-related mortality in the female population in Brazil[1,2]. It is also, probably, the type of cancer that causes the greatest fear in women because of its high occurrence and its psychological effects, which affect body image and quality of life[2]. Over the last 20 years, the techniques to treat breast cancer underwent significant changes. Nowadays, the surgeries are less invasive and the complementary therapeutic treatments (radiotherapy, chemotherapy and endocrine therapy) look to establish an adequate and balanced relationship between dose and secondary effects and dose and treatment efficiency.

Among surgical techniques, there are conservative ones, such as lumpectomy and quadrantectomy, and invasive ones, such as mastectomy [2-6]. Surgical procedures can determine immediate or subsequent physical complications, such as limitation of shoulder and elbow movement amplitude (MA),

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lymphedema, muscle weakness, infection, pain and paresthesia, sensitivity and functionality changes. Surgical procedures can damage the performance in daily physical activities, quality of life and women's tasks[2, 7-9].

It´s necessary an extensive evaluation of breast cancer treatment complications in order to adapt the best physiotherapeutic preoperative and postoperative follow-ups, as well as the best period for physiotherapeutic action.

Furthermore, a complete evaluation of these complications can support better decisions in future cases in which patients need treatment for breast cancer.

The goal of this study is to evaluate and compare body image perception, quality of life, tenderness alterations and pain presence and levels in women with breast cancer, during preoperative and postoperative periods of 30 (PO30), 60 (PO60), and 90 (PO90) days.

METHODOLOGY

We conducted a prospective longitudinal study from July 2011 to December 2013 on the perception of body image, quality of life, tenderness and pain in women diagnosed with breast cancer.

Sample

Women diagnosed with breast cancer who underwent tumor removal surgery were included. Individuals with cognitive defects that would prevent them from answering questionnaires, illiterate individuals, and individuals who did not agree with the terms of consent were excluded. Patients who did not continue to monitor the postoperative process and those who missed more than one evaluation during follow-up were also excluded. The patients were chosen at doctors' appointments at the Ambulatory of Onco-hematology of the Mastology discipline of the Gynecology Department of the Federal University of São Paulo - Unifesp.

Tools

The patients were informed about the research and signed a term of consent. After that, they answered some demographic questions and the following questionnaires: Evaluation Scale of body image "How I relate to my own body", EORTC QLQ-C30 and EORTC QLQ-BR23. The patients went through an evaluation of upper limb and breast tenderness using Monofilaments of Semmes-Weinstein from SORRI-BAURU. They were questioned on the presence and level of pain, from 0 to 10, according to the verbal numerical rating scale (VNRS).

The questionnaire "How I relate to my own body" evaluates a woman's body image, namely the premorbid body (value assigned to the body in general) and the morbid body (value assigned to a sick person's body). The answers are obtained through the Likert scale with an assertive base. The woman is questioned about the degree of agreement in relation to aspects of her body, such as how she relates to her body and how she values her appearance. The better the condition of the patient's body image, the higher the final score on the scale[10].

The EORTC QLQ C30 questionnaire is a general document referring to the quality of life with cancer. It has 30 questions that define the general quality of life, five functional scales (physical, performance, cognitive, emotional and social), three symptom scales (fatigue, pain and nausea/vomiting) and six single items (dyspnea, insomnia, loss of appetite, constipation, diarrhea and financial difficulties).

In addition, the section BR 23 from the EORTC QLQ questionnaire refers explicitly to the quality of life with breast cancer. This section has 23 questions presented on scales to measure side effects of chemotherapy, symptoms related to the upper limbs and breasts, body image and sexual function. It also includes single items that explore sexual satisfaction, hair loss distribution and future perspectives. All the score averages were transferred to a linear scale of 0 to 100 points, as described in the EORTC manual. The high scores of functional scales and overall quality of life represent, respectively, excellent function index and high quality of life, while high scores on scales of symptoms represent high levels of symptoms and problems[11].

The tenderness evaluation was performed with an esthesiometer, which helps evaluate and quantify the pressure threshold in the respective skin dermatomes and aids in detecting and monitoring nerve injuries. The esthesiometer used in the evaluation is called Monofilaments of Semmes-Weinstein by SORRI-BAURU. The assessment is made by following an order of predetermined colors produced by the manufacturer according to ply and strength (grams – g). It's possible to quantify sensitivity changes in the tested area through this order.

The pain evaluation was done with the VNRS, which quantifies pain intensity by numbers and ver-

bally refers to the patient. The VNRS is composed of 11 scores, 10 being the worst possible pain and 0 to 9 corresponding to different intermediate levels of pain. The patient can report verbally any number that they consider representative of what they are feeling[12].

Data collection procedure

During preoperative (PREOP) appointment, 30 days postoperative appointment (PO30), 60 days postoperative appointment (PO60), and 90 days postoperative appointment (PO90), the patients answered some questionnaires in order to verify body image perception and quality of life. They were submitted to a sensitivity evaluation in surgery areas, i.e. the breast area, inner area of the arm, and ipsilateral axilla, with an esthesiometer.

During the test, the patient was naked and sat with their back in a neutral position, with flexed elbows and hands on their waist. The nylon filament was placed perpendicular to the skin surface and lightly pressed until it began to bend. The contact between the filament and the skin was maintained for one and a half seconds, according to the manufacturer's recommendations. Then the patient was asked to report if they were feeling anything on their skin and the location of the feeling. When the patient felt nothing, the next color was used, and the test was redone.

At the end of the procedure, all patients were asked to verbally report if they felt any pain and its intensity on a score from 0 to 10.

Data analysis procedure

Microsoft Excel 2010 and Statistica 12 were used for data analysis tests on average, standard deviation, and percentages. Matched Wilcoxon Pairs Test Software was used when comparing PREOP and PO30, PREOP and PO60, and PREOP and PO90 considering a significance level of 0.05% ($p<0.05$).

RESULTS

A total of 180 women were evaluated. Twelve of them were excluded for not keeping up with the postoperative monitoring and two because of death. Thus, the study was composed of 166 patients with an average age of 59 years.

Regarding the evaluation of body image, we noticed that without PREOP most of the patients had an adequate body image perception and only a small portion had very low body image. All evaluations presented the same follow-up; only at PO30 was there a statistically significant difference.

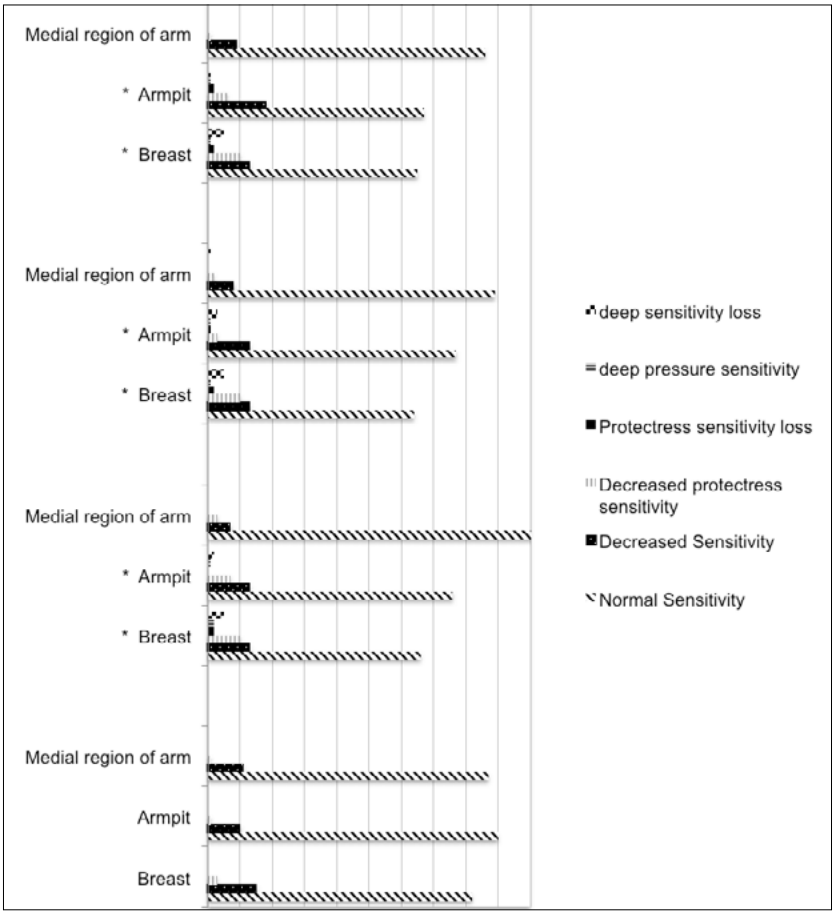
For the evaluation of the quality of life with the EORTC QLQ C30 questionnaire, we can see a significant alteration in Table I.

TABLE I. RESULTS OF EORTC QLQ C30. AVERAGE, STANDARD DEVIATION AND WILCOXON TEST ON A COMPARISON OF PREOP WITH OTHER PERIODS, CONSIDERING P<0,05

Periods	Average	Standard Deviation (±)	Comparison with PREOP by Wilcoxon test (p<0.05)
Physical Function			
PREOP	84.2	18.5	-
PO30	82.7	19.3	0.41
PO60	86.5	17.0	* 0.04
PO90	86.2	17.6	0.18
Emotional Function			
PREOP	62.1	29.9	-
PO30	71.2	28.7	* <0,001
PO60	73.6	29.2	* <0,001
PO90	71.9	28.8	* <0,001
Cognitive Function			
PREOP	77.9	26.2	-
PO30	82.1	25.8	* 0.01
PO60	79.6	26.1	0.48
PO90	79.9	27.4	0.61
Social Function			
PREOP	88.2	21.7	-
PO30	82.7	28.4	* 0.01
PO60	90.2	21.2	0.49
PO90	89.3	22.5	0.41
Nausea e Vomit			
PREOP	5.8	15.4	-
PO30	5.0	15.5	0.28
PO60	7.8	19.4	0.31
PO90	11.4	22.3	* 0.005
Appetite Loss			
PREOP	16.2	31.8	-
PO30	10.2	25.7	* 0.02
PO60	11.9	27.7	0.19
PO90	15.7	30.5	0.63
Constipation			
PREOP	20.2	35.1	-
PO30	26.6	38.0	0.06
PO60	22.8	35.1	0.36
PO90	27.4	38.7	* 0.03

Table I: ±: Standard deviation – the extension of deviation on the average; PREOP: the preoperative period before surgery; PO30: 30 days postoperative surgery; PO60: 60 days postoperative surgery; PO90: 90 days postoperative surgery *Wilcoxon test considering p< 0.05 in a comparison between PREOP and PO30/PO60/PO90.

CHART I. EVALUATION OF SENSIBILITY WITH ESTHESIOMETER



%: percentage of total number of patients; PREOP: preoperative period before surgery; PO30: 30 days postoperative surgery; PO60: 60 days postoperative surgery; PO90: 90 days postoperative surgery; *Wilcoxon test considering p< 0.05 in comparison between PREOP and PO30 – PREOP and PO60 – PREOP and PO90.

Table II shows statistically significant alterations using the EORTC QLQ BR23 for evaluation of the quality of life. We can see the sensitivity evaluation on Chart I.

The pain evaluation with VNRS showed a statistically significant increase at all periods, PO30, PO60, and PO90, with p<0.001 when compared to PREOP.

DISCUSSION

Several changes after breast cancer removal surgery can be seen at different times throughout the postoperative period. Thus, this type of cancer can affect women on a biopsychosocial scale[2].

Body image is considered a multidimensional phenomenon, because it involves physiological, psychological, and social aspects. It also affects emotion, thoughts, and the way people relate to each other[13-15].

Women go through an important process of reshaping their body image when they deal with breast cancer due to the various changes that disease and its treatment can cause to her body and mind [7,8,14,15].

This fact can be observed in our study, which showed a statistically significant decrease in body image at PO30 in comparison to PREOP. However, this study shows similar body image perception at preoperative evaluation and at the 60- and 90-day postoperative assessments. That similarity leads us to believe that patients tend to improve body image perception sometime after the surgery.

The complications from breast cancer treatment are often related to changes in the patient's quality of life[16]. In our study, we could see alterations in some scales of EORTC QLQ C30 and EORTC QLQ BR23. On EORTC QLQ BR23, the scales of breast cancer symptoms and arm symptoms presented better results at PO30 and PO60 when compared to PREOP.

Studies that evaluate the quality of life also saw

TABLE II. RESULTS OF EORTC QLQ BR23. AVERAGE, STANDARD DEVIATION AND WILCOXON TEST ON A COMPARISON OF PREOP WITH OTHER PERIODS, CONSIDERING P<0.05.

Period	Average	Standard Deviation(±)	Comparison with PREOP by Wilcoxon test (p=<0.05)
Body Image			
PREOP	84.5	23.4	-
PO30	80.6	26.8	* 0.02
PO60	86.4	22.6	0.65
PO90	83.6	25.4	0.7
Sexual Function			
PREOP	75.2	28.6	-
PO30	84.7	22.4	* <0.001
PO60	78.1	26.8	0.202
PO90	74.6	27.5	0.87
Sexual Satisfaction			
PREOP	41.9	37.0	-
PO30	62.4	34.5	* <0.001
PO60	45.3	33.8	0.13
PO90	45.8	34.8	0.13
Future Perspectives			
PREOP	44.4	40.5	-
PO30	52.0	40.3	* 0.02
PO60	57.3	38.2	* <0.001
PO90	57.7	40.2	* <0.001
Breast Symptoms			
PREOP	10.7	14.7	-
PO30	23.0	22.1	* <0.001
PO60	15.6	18.7	* 0.007
PO90	14.3	19.1	0.14
Arm Symptoms			
PREOP	11.2	18.6	-
PO30	20.5	20.6	* <0.001
PO60	16.8	21.3	* 0.003
PO90	15.5	20.8	* 0.01

Table II: Standard deviation – the extension of deviation on the average; PREOP: the preoperative period before surgery; PO30: 30 days postoperative surgery; PO60: 60 days postoperative surgery; PO90: 90 days postoperative surgery *Wilcoxon test considering p< 0.05 on a comparison between PREOP and PO30/PO60/PO90.

alterations in some aspects. Lahoz et al. [16], for example, saw more significant risks in the physical aspects, pain and vitality.

We also observed that the social function scale of EORTC QLQ C30 and the body image scale EORTC QLQ BR23 worsened significantly at PO30 compared to the preoperative period.

Confirming these results, some authors say that the topics of body image and quality of life can be profoundly influenced by medical issues. Surgical procedures can make a woman feel less attractive and start worrying about her body image. Surgery can also lead to loss of sensitivity on the breast area, intensifying changes in body image and woman's quality of life [13,17,18].

In our study, the scales of emotional function on the EORTC QLQ C30 and future perspectives on the EORTC QLQ BR23 had significantly better results in the three postoperative periods than in PREOP.

In addition, the scales of cognitive function and symptoms of loss of appetite on the EORTC QLQ C30 and the scales of sexual function and satisfaction on the EORTC QLQ BR23 got significantly better results at PO30 than at PREOP, and the scale of physical function was significantly better at PO60 than at PREOP. These facts can be justified by the same factor in Brandberg et al. [19], in which, due to tumor removal, the patient sees themselves free from the disease and improve in some aspects.

Sensitivity alterations also can be found in a significant number of women after breast cancer treatment [20].

We found worse results of sensitivity evaluation in this study, in which all the postoperative had a statistically significant alteration of p<0.001 in relation to PREOP.

Santos et al.[20] conducted a sensitivity evaluation with a Semmes-Weinstein esthesiometer on the intercostobrachial nerve in 94 women. The result showed a decrease of sensitivity in nerve dermatome intercostal ipsilateral after surgery.

Among all postoperative symptoms for breast cancer, pain is another common one. Pain in the arm and ipsilateral shoulder that persists six months or more after the surgical treatment has been reported in 25-60% of cases [21]. On postoperative, the present study showed pain in 45.8% of patients at PO30, 41.6% at PO60 and 39.7% at PO90, indicating an association with the information described above by Chiu et al. [21] and Ferreira et al.[22].

In the present study, it was possible to verify through the VNRS pain evaluation a statistically significant increase in all periods (PO30, PO60, and PO90) with p<0.001 when compared to PREOP. The increasing values mean the pain was higher, that is, the symptoms got worse during postoperative periods.

For Batiston and Santiago [23], pain has a multifactorial etiology and may be caused by nerve damage, psychological distress, reduced shoulder range of motion, muscle weakness and lymphedema.

In this study, sensitivity deficits as well as some aspects of quality of life and body image also present-

ed changes that can be related to pain. In the same way, Andrade et al. [24] mentioned that a negative body image could be associated with pain symptoms. However, even though our study didn't make a statistical comparison between pain and body image, these two topics are similar when monitored.

Lahoz et al. [16] mention complications such as lymphedema, pain, paresthesia, decreased muscle strength and decreased range of motion in the involved member are often observed and reported by women who had breast operations. These complications deserve attention because they interfere in the quality of life.

Thus, a multidisciplinary approach to breast cancer is necessary, in which physiotherapy must be focused on general prevention, mainly the maintenance or restoration of mobility and functional capacity of the arm, preventing the patient from adopting defensive positions and other complications.

In short, we can observe that several alterations caused by breast cancer treatment are connected, which means that each can cause the onset and worsening of the other. An evaluation of all aspects and scales of a patient with breast cancer has to be conducted at several points during treatment. Moreover, it is important to remember that the evaluator's and the patient's individual peculiarities must be considered in order to accurately diagnose and treat changes in the patient.

Through the use of questionnaires, it is possible to identify some aspects that would go unnoticed at a succinct evaluation. Although the instruments used are sometimes extensive, they are necessary because they help guide the clinical and multidisciplinary treatment of patients.

CONCLUSION

The patient's body image changed after breast cancer surgery. There were worse results at 30 days postoperative compared to the preoperative evaluation.

The quality of life was altered in some aspects measured by the EORTC QLQ C30 questionnaire. It was possible to see improved physical function, emotional function and cognitive function after the surgery. However, there was a significant decline in social function, loss of appetite, nausea symptoms and spew, and constipation. The body image, and breast and arm symptoms evaluated by the EORTC QLQ BR23 questionnaire deteriorated significantly at 30 days postoperative. The sexual satisfaction, function, and its perspectives presented better results at postoperative.

It was possible to notice significantly worse results in sensitivity and pain after surgery.

RESUMO

O câncer de mama é um dos tipos mais comuns de tumores no mundo e o tipo mais comum entre as mulheres. Existem tratamentos severos para o câncer de mama, no entanto, em muitos casos, podem ser acompanhados por complicações sérias para a vida da mulher. OBJETIVO: Avaliar e comparar a percepção da imagem corporal, a qualidade de vida, a sensibilidade e a dor em mulheres com câncer de mama nos períodos pré-operatório e pós-operatório de 30, 60 e 90 dias. MÉTODOS: Foi realizado um estudo longitudinal prospectivo. Os pacientes responderam ao questionário "Como me relaciono com meu próprio corpo", o EORTC QLQ-C30 e o EORTC QLQ-BR23. Fizemos uma avaliação da sensibilidade do membro superior e da mama com um estesiômetro. Os pacientes foram questionados sobre a presença de dor e seu nível em uma escala de 0 a 10. RESULTADOS: Para a imagem corporal, foi possível observar uma diferença significativa entre o pré e pós-operatório de 30 dias. Mostrou mudanças em algumas áreas dos questionários EORTC QLQ C30 e EORTC QLQ BR23, como sintomas de braço e mama, função social, constipação e função sexual e satisfação, entre outros. Para avaliação da sensibilidade mamária e axilar e avaliação da dor, todos os períodos de pós-operatório apresentaram diferenças significativas quando comparados ao período pré-operatório. A sensibilidade da região interna do braço não apresentou mudanças significativas. CONCLUSÃO: A diferença encontrada no estudo mostra que as avaliações em todas as escalas devem ser feitas em vários períodos, utilizando um tratamento adequado que enfrente as mudanças e a individualidade de cada paciente.

PALAVRAS CHAVE: *Sinais e sintomas. Modalidades de fisioterapia. Imagem corporal. Neoplasias da mama.*

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ORIGINAL ARTICLE

Religiousness is associated with lower levels of anxiety, but not depression, in medical and nursing students

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SUMMARY

OBJECTIVES: *To evaluate the association between religious and spiritual beliefs, anxiety and depression in medical and nursing students.*

METHODS: *A cross-sectional study was carried out with medical and nursing students from a Brazilian university. Students were randomly selected and filled out a questionnaire that contained sociodemographic, religious (Duke Religion Index), spirituality (Self-spirituality rating scale) and mental health - depression and anxiety (Hospital Anxiety and Depression Scale) data. Linear regression models were used to evaluate the association of R/E with mental health, with adjustments for sociodemographic variables.*

RESULTS: *A total of 187 students (90.7%) were included in the study, 56.1% female, an average of 23 years old, and 69% were enrolled in the medical program. Of the students, 29.4% attended religious services once a week or more often, 10.7% had private religious activities once a day or more often, and the indexes of intrinsic religiosity and spirituality were moderate. In the linear regression, adjusted for sociodemographic variables, the religious attendance was the only factor associated with lower levels of anxiety (Beta: -0.178, p=0.026). The other dimensions of religiousness or spirituality were not associated with levels of anxiety and depression.*

CONCLUSIONS: *The present study showed that only the religious attendance was associated with the mental health of the medical and nursing students. These results demonstrate that some students use religious support in an attempt to minimize the negative effects of their university life. This support seems to be more effective when it involves participation in religious social activities in relation to private activities.*

KEYWORDS: *Anxiety. Religion and Medicine. Students, Medical. Students, Nursing. Spirituality.*

INTRODUCTION

Starting university has an impact on people's lives, who are suddenly faced with new responsibilities that require a change in their habits, which may cause emotional conflicts[1]. Among the undergraduate programs, those in the health area are usually very demanding and their strenuous routine can be considered a stressful situation that can have a negative impact on student's well-being[3,4].

The adversity caused by academic difficulties, in addition to personal e social problems, may lead to emotional disorders. A systematic review including 40 studies showed that 13% to 25% of medical students suffer from depression and around 34% presented anxiety scores, evidencing a significant prevalence of these symptoms[5].

Faced with this, students may feel the need to develop coping mechanisms to deal with this new context of life[6]. Religiousness and spirituality are currently seen as strategies capable of providing an increased

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sense of purpose in life, well-being, and personal satisfaction, which are all associated with higher resistance to stressful situations[7-9]. Religiousness and spirituality are often used as synonyms. However, there is a growing distinction between these terms[1,2]. Religiousness is characterized by the experience of an organized system of beliefs and symbols shared between a group of people and presenting special behavioral, social, and doctrinal characteristics. It can be understood as organizational, non-organizational and intrinsic[10]. Spirituality is the personal search for answers and meaning to existential matters, involving the understanding of the purposes of life and its possible connections with transcendence[11].

Religiousness is being increasingly studied nowadays[12-15].

In a systematic review conducted between 2003 and 2013, it was evidenced that most studies pointed out that spiritual/religious activities play a protective role in mental health and the prevalence of minor psychiatric disorders (anxiety and depression)[16]. By means of spirituality, religiousness, or both, people can positively associate mental and physical well-being dimensions, thus experiencing a sense of shelter when faced with adversity[17]. It is also known that both spirituality and religiousness influence stress reduction and are therefore considered protective factors for depression and anxiety disorders[18,19].

Despite the evidence, there are still few studies that have assessed how religious beliefs influence the mental health of medical students and their results are conflicting[9,20,21].

This way, this study aims to evaluate the connection between spiritual/religious beliefs, anxiety, and depression in medical and nursing students.

METHODOLOGY

This is a cross-sectional observational study with a quantitative approach conducted in the Federal University of the Triângulo Mineiro (UFTM), in Uberaba, Minas Gerais, Brazil, during November and December of 2015. It was authorized by the Research Ethics Committee of the Federal University of the Triângulo Mineiro by decision No 1.235.044, and the students signed an informed consent form in order to participate.

UFTM is a Brazilian public university that has approximately 5,000 students and programs on several knowledge areas, including in health. The medical (486 students enrolled) and nursing (271 students enrolled) programs have a student-focused traditional curriculum.

For this study, we included students enrolled in the medical and nursing programs that were 18 years old or older, both male and female, who voluntarily accepted to be included. According to a previous sample calculation, we needed 206 students, who were randomly drawn using a list with the names of all students. The students were then approached in person by previously trained research collaborators, before or after classes, in pre-established moments, when they were informed of the purpose of the study.

The questionnaire was self-filling, took 15 minutes and was composed of:

- Sociodemographic variables: age, gender, ethnicity and family income.
- Religiousness: by means of the Duke Religion Index (Durel). It is a brief multidimensional measurement that uses 5 items to measure three dimensions of religious involvement: organizational (ORA) - attendance at religious meetings, such as study groups, cults, and masses; non-organizational (NORA) - frequency at private religious activities, such as prayer, meditation, and reading of religious texts; and intrinsic religiousness (IR) - Search for internalization and complete religious experience as the individual's main objective. Immediate ends are considered secondary and achieved in harmony with basic religious principles. The scale is authored by Koenig et al. (2001) and has been validated in Portuguese[22].
- Spirituality: using the Spirituality Self Rating Scale (SSRS), which consists of a scale that assesses aspects of spirituality with items that focus on the individual spiritual orientation, whether the individual considers spiritual matters important or not, and how this applies to their lives. It is a self-filling instrument composed of six items. The original scale was developed by Galanter at al. and was validated into Portuguese[23].
- Hospital Anxiety and Depression Scale (HADS): is a scale that consists of two subscales with 14 multiple choice questions, 7 for anxiety and 7 for depression. The overall score in each subscale ranges from 0 to 21. The scale was developed by Zigmond and Snaith in 1983 and validated into Portuguese[24]. Despite having been

initially created for non-psychiatric hospitalized patients, it has been currently used in literature for several other populations, including medical students[25].

The sample size calculation corresponded to the proportional number of each program, considering an additional 25% of losses. It considered a determination coefficient R^2=0.10 in a multiple linear regression model with five predictors (R/E variable and sociodemographic), with type I error or significance level Alfa=0.05, and type II 0,10, thus resulting in a priori statistical power of 90%. The sample was made up of 206 students total, 131 from the medical program, and 75 from the nursing program. The students selected were contacted, and those who agreed to participate individually filled out the printed questionnaires on a date and time arranged between them and the researcher in charge.

The data consistency was performed by double-entry typing on an Excel sheet. Next, they were exported to the SPSS (Statistical Package for the Social Science) application, version 20.0, for conducting the statistical analysis. The data was analyzed by descriptive techniques, frequency distribution, mean and standard deviation.

The inferential analysis used the Student's t-test for comparing anxiety scores and the categorical variables (program and gender). Then, linear regression models were performed with the HADS scores for depression and anxiety as dependent variables; R/E measures were added individually and adjusted for sociodemographic variables (gender, age, ethnicity and family income). We chose not to group all the religiousness and spirituality variables into a single model due to the high correlation between them (above r = 0.60), which would increase multi-

TABLE 1 - PERCENTAGE DISTRIBUTION ON RELIGIOUSNESS OF MEDICAL AND NURSING STUDENTS FROM A UNIVERSITY IN THE MUNICIPALITY OF UBERABA/MG, 2015.

Variables		N	%
Religion	Catholic	68	36.7
	Protestant	7	3.7
	Adventist	3	1.6
	Evangelical	13	7
	Spiritualist	29	15.5
	Others	3	1.6
	Unfilled	64	34
How often do you attend church or other religious meetings?	More than once a week	15	8.2
	Once a week	37	19.7
	Two or three times per month	27	14.4
	A couple of times a year	44	23.5
	Once a year or less	35	18.7
	Never	29	15.5
How often do you dedicate your time to individual religious activities such as prayer, meditation, reading the Bible or other religious texts?	More than once a day	12	6.4
	Daily	63	33.7
	Two a week or more	23	12.3
	Once a week	20	10.7
	A few times per month	25	13.4
	Rarely or never	44	23.5
I feel the presence of God (or the Holy Spirit) in my life.	Completely true for me	85	45.4
	Mostly true	53	28.3
	I am not sure	23	12.3
	Mostly not true	7	3.7
	Not true	19	10.2
My religious beliefs are the foundation of my entire way of living.	Completely true for me	27	14.4
	Mostly true	63	33.7
	I am not sure	31	16.6
	Mostly not true	26	13.9
	Not true	40	21.4
I make a real effort to put my religion into practice in all aspects of my life.	Completely true for me	24	12.8
	Mostly true	56	29.9
	I am not sure	32	17.2
	Mostly not true	31	16.6
	Not true	44	23.5

Source: Prepared by the authors.

collinearity. A $p < 0.05$ was determined as significant and Beta values were described, as well as the proportion of variance in the dependent variable in relation to the predictor variables (R-square).

RESULTS

In the sample of 206 students, 187 (90.7%) agreed to participate in the research. Of these, 69% were medical students and 31% nursing students, most of them between 18 and 44 years old, with an average age of 23. As for gender, 56.1% were females and 43.9% males; 67.9% declared themselves to be white. When questioned about religion, 27.9% said to attend religious services once a week or more often, 40.1% engaged in private religious practices once a day or more often, and 34.8% declared not having any religion (Table 1).

The scores from the anxiety and depression questionnaire were bivariate compared by means of the Student's t-test regarding program, gender, and ethnicity (Table 2) For male individuals, the mean anxiety was 7.36 (DP: 3.89), in females it was 8.57 (SD: 3,99), with statistical relevance in the comparison between them. When comparing anxiety scores and program, no statistical relevance was found (Table 2).

None of the religiousness and spirituality variables were associated with the levels of depression in the sample studied, in both adjusted and non-adjusted models. Regarding anxiety, only organizational religiosity (religious attendance) was associated with lower levels of anxiety (Beta=-0.178, p=0.026) in the adjusted model. Despite the statistical significance, the proportion of variance in the dependent variable in relation to the predictor variables (R-square) was low (R-square=0.005) for the model without adjustment and (R-square=0.065) for the adjusted model (Table 3).

DISCUSSION

The present study showed that medical and nursing students have religious and spiritual beliefs, and that only religious attendance was associated with fewer anxiety symptoms, but the same did not apply to depression. These data highlight the fact that even among young people with high levels of education

TABLE 2 - COMPARISON OF ANXIETY AND DEPRESSION SCORES BY PROGRAM, GENDER, AND ETHNICITY OF THE MEDICAL AND NURSING STUDENTS FROM A UNIVERSITY IN THE MUNICIPALITY OF UBERABA/MG, 2015.

	Mean anxiety	Anxiety standard deviation	Anxiety P	Mean depression	Depression standard deviation	Depression P
Program			0.98			0.305
Medicine	8.04	4.01		5.44	3.28	
Nursing	8.03	3.94		4.93	2.82	
Gender			0.04			0.047
Female	8.57	3.99		5.68	3.09	
Men	7.36	3.89		4.77	3.15	

Source: Prepared by the authors.(Student's t-test, $p \leq 0.05$)

TABLE 3 - LINEAR REGRESSION ASSESSING THE FACTORS ASSOCIATED WITH ANXIETY AND DEPRESSION IN MEDICAL AND NURSING STUDENTS FROM A UNIVERSITY IN THE CITY OF UBERABA/MG, 2015

	Non-adjusted regression				Regression adjusted for sociodemographic characteristics			
	B (SE)	Beta	p	Model R-square	B (SE)	Beta	p	Model R-square
Depression								
Spirituality	-0.017 (0.036)	-0.036	0.629	0.001	-0.012 (0.038)	-0.025	0.750	0.054
ORA	-0.052 (0.149)	-0.025	0.730	0.001	-0.217 (0.162)	-0.107	0.181	0.063
NORA	-0.050 (0.133)	-0.027	0.710	0.001	-0.036 (0.138)	-0.020	0.791	0.054
IR	-0.028 (0.063)	-0.033	0.653	0.001	-0.029 (0.068)	-0.033	0.673	0.055
Anxiety								
Spirituality	-0.004 (0.045)	-0.006	0.937	0.001	-0.046 (0.048)	-0.075	0.340	0.044
ORA	-0.173 (0.189)	-0.067	0.360	0.005	-0.459 (0.205)	-0.178	0.026	0.065
NORA	0.025 (0.169)	0.011	0.882	0.001	-0.120 (0.175)	-0.052	0.496	0.041
IR	0.049 (0.080)	0.045	0.545	0.002	0.039 (0.087)	0.036	0.654	0.040

*p < 0.05 **p < 0.01 ***p < 0.001 Model 1: gender, age, ethnicity, income.

religious beliefs are still used as a possible coping mechanism for adverse situations.

Comparing our findings with those of other institutions, they are similar to those of Lupo and Strous[21], who found that religiousness was associated to lower levels of anxiety symptoms in Israeli medical students, and of Vasegh and Mohammadi[20], who verified that religious belief was associated to less anxiety in Iranian medical students. However, our findings differ from those of another study that found a connection between religiousness and depression, but not anxiety, in Brazilian medical students[9].

These sometimes discordant results can be justified by a number of factors. Religious affiliation and culture can change depending on the type of sample studied; institutions can also have different curriculum and offer different types of psychological support; and religiousness can often have a more negative (religious suffering) or positive nature. In a recent systematic review, Koenig[26] evaluated 299 studies and found that 147 of them (49%) showed an inverse association between R/E and anxiety (a result compatible with our findings), while 127 found no connection, and 33 showed a positive association. The same results were found for depression. The author found that of the 444 studies examining the connection between R/E and depression, 272 (61%) indicated an inverse association, 144 found no connection, and 28 found a positive association.

In the same way that the association between R/E and anxiety can vary among studies, the religiousness measurements considered (ORA, NORA, IR) also present variance in the literature. In a study by Luchetti et al. that assessed the presence of anxiety in nurses that worked in home care, it was found that those who presented higher anxiety levels were the ones with lower ORA and higher NORA. In another study conducted with adults in a Malaysian community, it was found that IR and NORA behaved as possible risk factors for anxiety[28]. Religiousness as a risk factor was also demonstrated by Mohamad et al[29].

From the studies presented, we can infer that the religious measurements can sometimes behave as risk factors, and other times as protective factors, under different situations and varying according to the context and population. According to Moutinho et al[9], it is important to have studies that assess mental health and its relationship with religiousness in different groups, such as university students, aiming at the early identification of risks and a greater understanding of the relationship between religiousness and adversity. This can lead to the development of prevention and coping mechanisms specific to each population.

Finally, our findings reinforce the premise that students use their beliefs as a way of dealing with adversity, which is corroborated by other studies with different populations in which religion offers a coping mechanism for stressful stuations[7,8], such as disease-related vulnerability[30], the emotional impact felt when faced with the brevity of life[31], and the difficulties inherent to health care[32]. Educators must be aware of the students' needs and of how religiousness and spirituality are used (functionally or dysfunctionally).

As limitations of the study, we can mention the use of two health care courses, since nowadays there is a tendency to have multidisciplinary teams in health care. Additionally, there is the fact that a cross-sectional approach was used, when a longitudinal follow-up could clarify possible variations in the relationship between spirituality, religiousness and anxiety throughout the program. Finally, the students' social support was not evaluated. Religious attendance can lead to a greater social support, which can be a mechanism for better mental health.

CONCLUSION

The present study showed that only the religious attendance was associated with the mental health of medical and nursing students, who used the religious practice to minimize the negative impacts of university life. This support seems to be more effective when it involves the participation in social religious activities over private activities.

In the academic environment, the possible impact of religiousness on anxiety levels requires new studies focused on understanding the perception of students regarding these themes for a broader understanding of their relationships. In addition, in the healthcare perspective, the importance of belief when faced with sickness has been increasingly acknowledged, which highlights the importance given to spirituality and religiousness by patients, family members and staff. In this way, it is necessary that future professionals be fully prepared in order to have the required sensitivity to fully exercise the abilities learned at the university as a perception tool, knowing how to deal with the spirituality and religiousness of each individual.

RESUMO

OBJETIVO: *Avaliar a associação entre crenças religiosas/espirituais, ansiedade e depressão em estudantes dos cursos de medicina e enfermagem.*

MÉTODOS: *Estudo transversal realizado com estudantes de medicina e enfermagem de uma universidade brasileira. Estudantes foram sorteados de forma aleatória e preencheram um questionário que continha dados sociodemográficos, de religiosidade (*Duke Religion Index*), espiritualidade (*Self-spirituality rating scale*) e saúde mental – depressão e ansiedade (Hospital* ***Anxietyand Depression Scale****). Foram usados modelos de regressão linear para avaliar a associação de R/E com saúde mental ajustando para as variáveis sociodemográficas.*

RESULTADOS: *Um total de 187 estudantes abordados (90,7%) foi incluído no estudo, sendo 56,1% do sexo feminino, média de 23 anos de idade e 69% fazendo parte do curso de medicina. Dos estudantes, 29,4% frequentavam serviços religiosos uma vez ou mais por semana, 10,7% realizavam atividades privadas religiosas uma ou mais vezes ao dia e os índices de religiosidade intrínseca e espiritualidade foram moderados. Na regressão linear, ajustada para variáveis sociodemográficas, a frequência religiosa foi a única que esteve associada a menores níveis de ansiedade (Beta: −0,178, p = 0,026). As demais dimensões de religiosidade ou espiritualidade não estiveram associadas a níveis de ansiedade e depressão.*

CONCLUSÕES: *O presente estudo evidenciou que apenas a frequência religiosa esteve associada à saúde mental do estudante de medicina e enfermagem. Esses resultados demonstram que alguns estudantes utilizam suporte religioso na tentativa de minimizar os efeitos negativos de sua vida universitária. Esse suporte parece ser mais efetivo quando envolve a participação em atividades sociais religiosas em relação a atividades privadas.*

PALAVRAS-CHAVE: *Ansiedade. Religião e medicina. Estudantes de medicina. Estudantes de enfermagem. Espiritualidade.*

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ORIGINAL ARTICLE

Benign versus life-threatening causes of pneumatosis intestinalis: differentiating CT features

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SUMMARY

OBJECTIVE: *To assess the diagnostic performance of CT findings in differentiating causes of pneumatosis intestinalis (PI), including benign and life-threatening causes.*

METHODS: *All CT reports containing the word "pneumatosis" were queried from June 1st, 2006 to May 31st, 2015. A total of 42 patients with PI were enrolled (mean age, 63.4 years; 23 males and 19 females) and divided into two groups on based on electronic medical records: a benign group (n=24) and a life-threatening group (n=18). Two radiologists reviewed CT images and evaluated CT findings including bowel distension, the pattern of bowel wall enhancement, bowel wall defect, portal venous gas (PVG), mesenteric venous gas (MVG), extraluminal free air, and ascites.*

RESULTS: *CT findings including bowel distension, decreased bowel wall enhancement, PVG, and ascites were more commonly identified in the life-threatening group (all p<0.05). All cases with PVG were included in the life-threatening group (8/18 patients, 44.4%). Bowel wall defect, extraluminal free air, and mesenteric venous gas showed no statistical significance between both groups.*

CONCLUSION: *PI and concurrent PVG, bowel distension, decreased bowel wall enhancement, or ascites were significantly associated with life-threatening causes and unfavorable prognosis. Thus, evaluating ancillary CT features when we encountered PI would help us characterize the causes of PI and determine the appropriate treatment option.*

KEYWORDS: *Pneumatosis cystoides intestinalis. Pneumoperitoneum. Intestinal perforation. Mesenteric ischemia.*

INTRODUCTION

Pneumatosis intestinalis (PI) is a radiographic or physical finding characterized by gas infiltration into the wall of the intestine. The clinical significance of PI can vary as it is the result of benign or life-threatening medical conditions and also can be an incidental finding[1-3]. Although the pathophysiology of PI remains unclear, three mechanisms have been proposed as the cause of intestinal wall gas: (1) intraluminal gas entering the bowel wall through mucosal breaks, which may cause gas spread along the mesentery[4,5]; (2) luminal bacteria producing excessive amounts of hydrogen gas, causing intestinal luminal pressure increase and resulting in directly-forced gas trapped within the submucosa[6,7]; and (3) pulmonary gas from alveolar rupture, coursing through the mediastinum to the retroperitoneum and mesentery[8].

PI is traditionally considered a sign of bowel wall infarction and a surgical emergency, especially in

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cases associated with portomesenteric venous gas[9] or pneumoperitoneum[1,2,10]. The mortality rates of PI are reported 65%-86% in previous studies when accompanied by portal venous gas (PVG)[11-14]. However, recently numerous non-ischemic causes of PI have been described because of the improved sensitivity in detection of PI by computed tomography (CT): non-ischemic causes of PI include inflammatory bowel disease, intestinal dilatation, connective tissue disease, organ transplantation or post-operative change, immune-deficiency status, and chemotherapy [15-19]. PI induced by these non-ischemic causes commonly show a benign clinical course and require conservative management rather than surgery. Due to the increased incidence of PI and an increased number of causes, including both life-threatening and benign ones, it is still confusing to select the most appropriate treatment option in clinical practice. Furthermore, sometimes surgical intervention is unnecessary and even harmful. Thus, an evaluation for the specific cause of PI is clinically important to reduce unnecessary surgery, leading to improved clinical outcomes of the patients.

The purpose of this study is to assess the diagnostic performance of the CT findings in the characterization of causes of PI, including benign and life-threatening causes.

METHODS

Subjects

This study was approved by our institutional review board, which waived the need for informed consent. The CT scan database of the radiologic department was queried for all reports containing the word "pneumatosis" from June 1st, 2006 to May 31st, 2015. Among the selected reports, we secondarily looked for reports which included any of the following terms: "pneumoperitoneum", "pneumoretroperitoneum", "free air", or "extraluminal air". We then confirmed the report findings by review of CT scan images. Repeated CT scans on the same patient were excluded from the analysis. Finally, 42 consecutive patients with PI were enrolled (mean age, 63.4 years; range, 30-91 years; 23 males and 19 females). We assessed their clinical status at the time of the CT scan by reviewing their electronic medical records (EMR), including their vital signs and the presence of clinical symptoms, such as abdominal pain. The clinical course and management were also assessed based on the EMR. The enrolled patients were divided into two groups by reviewing clinical reports on EMR that were written on the same day or prior to the CT scan: The benign group, n=24, had no symptom complaints or minimal symptoms, including abdominal discomfort, and the possible cause of PI was considered as a benign disease entity; the life-threatening group, n=18, presented severe abdominal pain or unstable vital signs.

Image analysis

Thirty-seven patients were examined using a 64-detector CT scanner (Sensation 64; Simens Medical System, Erlangen, Germany), the other 5 patients underwent other CT scanners (two patients, Sensation 4, Simens Medical System, Erlangen, Germany; two patients, GE Discovery CT 750HD, GE Healthcare, Waukesha, WI, USA; and one patient scanned using Light Speed VCT, GE Healthcare, Waukesha, WI, USA). Intravenous contrast media were used in most of the patients and only two of them underwent a non-enhanced scan due to poor renal function.

Two radiologists (a board-certified abdominal radiology expert with 10 years of experience and a radiology expert with 3 years of experience) reviewed all CT images independently. The readers were blinded to the clinical diagnosis of the enrolled patients and evaluated the following CT findings: pattern of bowel wall enhancement, presence of bowel distension or bowel wall defect, extraluminal free air (pneumoperitoneum or pneumoretroperitoneum), portal venous gas (PVG) or mesenteric venous gas (MVG), and the presence of ascites. The pattern of bowel wall enhancement was categorized as decreased and normal and was determined by comparison with that of the adjacent bowel wall. The MVG was determined when extraluminal gas appeared as a linear or curvilinear shape along the mesenteric border of bowel loops, especially the bowel segment showing PI (Fig.1).

Statistics

All statistical analyses were performed using SPSS for Windows version 15.0 (SPSS Inc., Chicago, IL, USA). Results were expressed as mean ± SD unless stated otherwise. A comparison of the CT findings between both groups was performed using Fisher's exact test. A P value inferior to 0.05 was considered to be statistically significant.

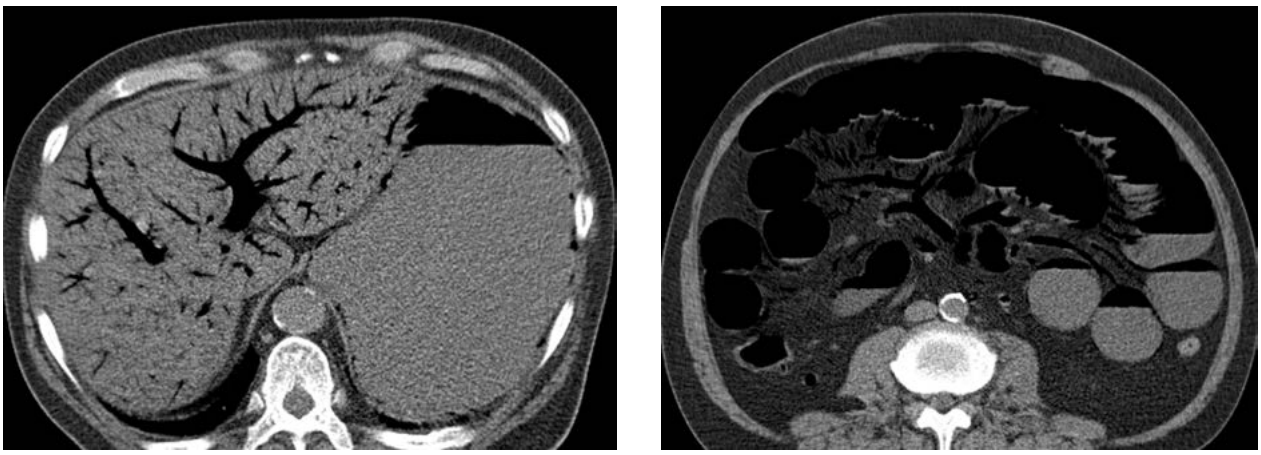

FIG. 1. A 59-YEAR-OLD MALE WHO PRESENTED ACUTE ABDOMINAL PAIN.
A. Axial precontrast CT image shows diffuse small bowel distension with PI and extensive MVG. B. Extensive PVG is also shown on precontrast CT scan. Decreased bowel wall enhancement was also identified, probably as a result of extensive bowel ischemia in this case. This patient experienced sudden cardiac arrest immediately after CT scanning and finally expired.

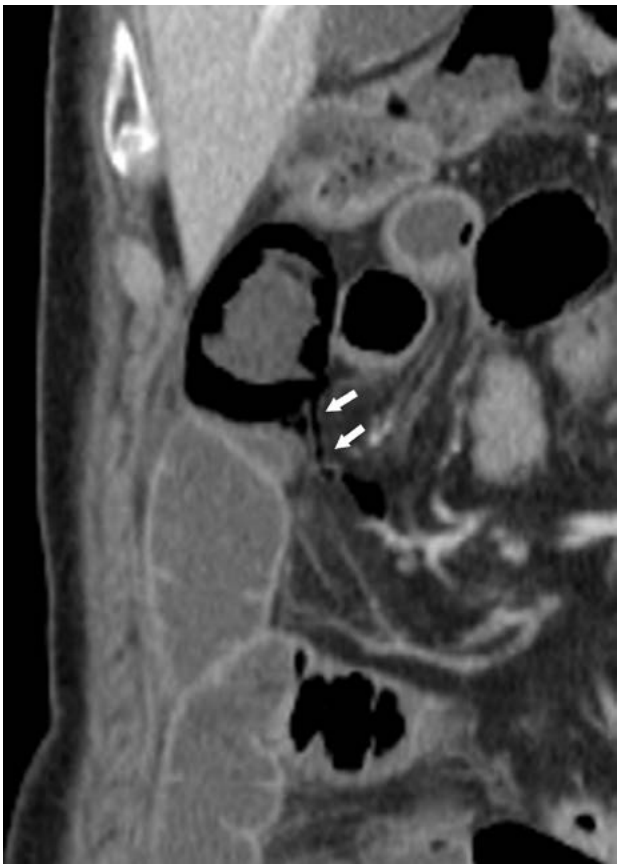

FIG.2. A 76-YEAR-OLD MALE WHO PRESENTED ABDOMINAL PAIN.
Coronal reformatted contrast-enhanced CT image shows PI in jejunal loops and an associated small amount of mesenteric venous gas (white arrow). This patient underwent conservative treatment and the abdominal pain subsided. PI was also resolved on follow up abdomen CT scan.

RESULTS

CT findings

Results of the comparison of CT measurements between both patient groups are summarized in Table 1. The CT findings including bowel distension, decreased bowel wall enhancement, PVG, and ascites were significantly more commonly identified in the life-threatening group (all $p<0.05$). The life-threatening group showed more frequent bowel distension and decreased bowel wall enhancement with statistical significance (bowel distension, 20.8% vs. 83.3%, $p<0.001$; and decreased bowel wall enhancement, 4.5% vs. 77.8%, $p<0.001$). All cases with PVG were included in the life-threatening group (Fig.1) (8/18 patients, 44.4%, $p<0.001$). Ascites was more frequently detected in the life-threatening group (13/18 patients, 72.2%) than in the benign group (7/24 patients, 29.2%) and the result was statistically significant ($p=0.012$). No significant correlation was seen between both patient groups in the analysis of CT findings, such as bowel wall defect, extraluminal free air, and MVG (Fig.2). Of all 42 patients, 2 with bowel wall defect were identified and included in the life-threatening group without clinical significance ($p=0.196$). MVG was detected in both two patient groups but with no statistical significance (benign group, 8/24 patients, 33.3%; and life-threatening group, 12/18 patients, 66.7%; $p=0.060$).

Patient Outcome

The benign group (n=24) reported no symptom or improvement of symptoms with the resolution of the detected CT findings on follow-up abdominal radiography or CT scan. None of them had any specific

TABLE 1. CT MEASUREMENTS AND COMPARISON IN PATIENT GROUPS

	Benign group n=24	Life-threatening group n=18	p value
Bowel distension	5	15	.000
Decreased bowel wall enhancement	1*	14	.000
Bowel wall defect	0*	2	.196
Extraluminal free air	13	11	.757
PVG	0	8	.000
MVG	8	12	.060
Ascites	7	13	.012

Note- Data are the number of patients and (%), PVG=portal venous gas, MVG=mesenteric venous gas. *The total number of patients is 22 because two patients with nonenhanced abdomen CT scan in benign group were excluded.

TABLE 2. PATIENT OUTCOME

Benign group		
	Conservative treatment, n=24	
Life-threatening group		
	Death, n=1	
	Indicated to surgical intervention, n=13	
		Undergo emergent surgery, n=9
		Refused to undergo surgery, n=4
	Endoscopic examination proven duodenal ulcer and clipping, n=1	
	Antibiotics therapy and intensive care, n=3	

medical or surgical intervention. The life-threatening group (n=18) was recommended surgical intervention at the presentation due to unstable vital signs or hospitalization with close observation. Of the patients in the life-threatening group, 9 underwent emergency surgery due to bowel ischemia, 1 died immediately after taking the CT scan due to bowel ischemia, 4 were recommended for surgical intervention but refused, 3 underwent antibiotics therapy for ischemic bowel disease and were discharged with improved status, and 1 had duodenal ulcer bleeding and improved clinical status after endoscopic bleeder clipping. The patient outcome of all enrolled patients is summarized in Table 2.

Discussion

PI is traditionally considered a surgical emergency with a high possibility of bowel ischemia, especially in cases associated with portomesenteric venous gas. However, previous studies reported that PI might occur after infection or inflammation, ulceration, surgery or trauma[20,21]. In addition, the incidence of asymptomatic PI has been increasing in association with the development of CT scanning.

In this study, PVG had statistical significance and was only identified in the life-threatening group. However, several studies[22,23] have reported that the PVG is not a useful indicator of bowel ischemia and is not helpful in determining the need for surgical intervention. Faberman et al.[22] analyzed 17 patients with PMVG on CT and reported a 71% survival rate. The different result can be associated with the different study design, as they enrolled patients with PMVG and only 9 of all 17 patients had combined PI. In our study, we enrolled a larger number of patients and all of them had PI. Additionally, all of the patients with PVG were included in the life-threatening group with statistical significance. This result supports other previous larger studies[2,24-26], which suggested that PI combined with PVG is associated with severe mesenteric ischemia and unfavorable clinical outcomes.

Regarding MVG, which we suspected to have similar significance when compared with PVG, it was analyzed aside of PVG. MVG was identified in about half of the patients (47.6%) but had no statistical significance. To our knowledge, no one analyzed the incidence and significance of MVG in association with PI. The result suggests that, even though the cause of MVG is unknown, we could identify it in a relatively high incidence. Thus, we can presume MVG itself is not an ominous sign. However, when considering the statistical significance of PVG, we need further studies to analyze the association of MVG and PVG with a

separate measurement of both CT findings.

Bowel distension and ascites were significantly more commonly identified in the life-threatening group. Concurrent bowel distension and ascites are known to be associated with high-grade obstruction and congestion. In this study, decreased bowel wall enhancement, which is a radiologic indicator of bowel ischemia, was also significantly associated with the life-threatening group. The result is similar to that of previous larger studies[25,27]. Duron et al.[27] analyzed radiologic findings of 150 patients diagnosed with PI on CT and compared non-operative and operative groups; dilated bowel loops and free fluid were significantly associated with the operative group. In a study by Lee et al.[25], that analyzed 123 patients with PI, decreased or absent enhancement of the bowel wall on CT were associated with increased mortality. Therefore, patients with PI and bowel distension or decreased bowel wall enhancement or ascites should be observed vigilantly.

Extraluminal free air, including both pneumoperitoneum and pneumoretroperitoneum, has been considered a sign of perforated hollow viscus and weighted heavily in favor of surgical management. However, in a previous study, it was suggested that pneumoperitoneum could occur with long-standing PI and rarely is associated with peritonitis[28]. In this study, extraluminal free air did not significantly correlate with patient outcome, and even the benign group presented extraluminal free air in about half of the patients (54.2%). On the other hand, bowel wall defect on a CT scan, which is a direct indicator of perforated hollow viscus, was identified in only two patients among a total of 42 patients, with no statistical significance. In this study, two cases with both PI and bowel wall defect resulted from transmural bowel infarction: one patient had colon infarction and underwent emergent segmental resection of the colon; and the other expired immediately after taking the CT scan due to extensive small bowel ischemia. However, the rest of the life-threatening group showed no significant bowel wall defect, even though about two-thirds of them showed extraluminal free air. This result may support that pneumoperitoneum with PI itself is not an ominous sign, so it is best to look for other critical signs such as bowel wall abnormality.

There were several limitations to the study. First, because this study was conducted at a tertiary referral center, there is a selection bias. Second, it presents a retrospective study design, so there is a possibility of insufficient clinical information. Third, about two-thirds (66.7%) of the patients were managed nonoperatively, so it was not possible to confirm the presence or absence of bowel ischemia or other pathologic findings in both groups.

CONCLUSION

It is still difficult to determine the management of patients with PI because there are various interpretations of the clinical significance of PI and its associated CT findings.

This study revealed that the PI and concurrent PVG, bowel distension, ascites and decreased bowel wall enhancement were significantly associated with life-threatening causes of PI and unfavorable clinical outcomes. On the other hand, the presence of MVG, extraluminal free air, and bowel wall defects showed no statistical significance. Thus, it is necessary to pay attention to other ancillary CT findings when interpreting images of patients with PI to help characterize the causes of PI and determine the appropriate treatment option.

RESUMO

OBJETIVO: *Avaliar o desempenho diagnóstico dos achados CT em causas diferenciadoras da pneumatose intestinal (PI), incluindo causas benignas e que ameaçam a vida.*

MÉTODOS: *Todos os relatórios CT contendo a palavra "pneumatose" foram questionados de 1º de junho de 2006 a 31 de maio de 2015. Um total de 42 pacientes com PI foi matriculado (idade média 63,4 anos, 23 do sexo masculino e 19 do sexo feminino) e divididos em dois grupos na base de registros médicos elétricos: grupo benigno, n = 24 e grupo com risco de vida, n = 18. Dois radiologistas analisaram as imagens da CT e avaliaram seus achados, incluindo distensão intestinal, padrão de realce da parede intestinal, defeito da parede intestinal, gás venoso portal (PVG), gás venoso mesentérico (MVG), ar extraluminal e ascite.*

RESULTADOS: *Achados CT, incluindo distensão intestinal, diminuição do realce da parede intestinal. PVG e ascite foram mais comumente identificados em grupo com risco de vida (todos p < 0,05, respectivamente). Todos os casos com PVG foram incluídos em grupo com risco de vida (8/18 pacientes, 44,4%). Defeito da parede do intestino, ar livre extraluminal e gás venoso mesentérico não mostraram significância estatística entre dois grupos.*

CONCLUSÃO: *PI e PVG concorrente, distensão intestinal, diminuição do aumento da parede do intestino ou ascites foram significativamente associados com causas que ameaçaram a vida e prognóstico desfavorável. Portanto, avaliar os recursos de CT auxiliares quando encontramos PI nos ajudaria a caracterizar as causas de PI e determinar a opção de tratamento apropriada.*

PALAVRAS-CHAVE: *Pneumatose cistoide intestinal. Pneumoperitônio. Perfuração intestinal. Isquemia mesentérica.*

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ORIGINAL ARTICLE

Relationship of the skin and subcutaneous tissue thickness in the tensiomyography response: a novel ultrasound observational study

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SUMMARY

BACKGROUND: *The aim of the study was to describe and correlate the skin, subcutaneous tissue, and superficial fascia thickness assessed by ultrasonography (US) with the lumbar erector spinae muscles contractile properties evaluated by tensiomyography (TMG).*

METHODS: *A cross-sectional descriptive study with 50 healthy participants was performed. The point of maximum lordosis in the lumbar region of the right erector spinae was evaluated by US and TMG. First, the skin, subcutaneous tissue, and superficial fascia thicknesses (cm) were assessed by US. Second, the five contractile TMG parameters were analyzed from the right erector spinae muscles belly displacement-time curves: maximal radial displacement (Dm), contraction time (Tc), sustain time (Ts), delay time (Td), and half-relaxation time (Tr). Finally, correlation analyses using Pearson (**r** for parametric data) and Spearman (r_s for non-parametric data) coefficients were performed.*

RESULTS: *A strong negative correlation was shown between Dm and subcutaneous tissue thickness (r_s=-0.668; P<.001). Furthermore, moderate negative correlations were observed between Dm and skin thickness (r=-0.329; P=0.020) as well as Tr and subcutaneous tissue thickness (r_s=-0.369; P=0.008). The rest of the parameters did not show statistically significant correlations (P > .05).*

CONCLUSION: *Therefore, the lumbar erector spinae contractile properties during TMG assessments, especially Dm and Tr, may be widely correlated by the skin and subcutaneous tissue thickness.*

KEYWORDS: *Muscle contraction. Skin. Subcutaneous tissue. Ultrasonography.*

ABBREVIATIONS: *BMI, Body Mass Index; Dm, maximal radial displacement; **r**, Pearson correlation coefficient; r_s, Spearman correlation coefficient; Tc; contraction time; Ts, sustain time; Td, delay time; Tr, half-relaxation time; TMG, tensiomyography; US, ultrasonography.*

INTRODUCTION

The structural properties, such as deformation, thickness and hardness, of the skin, subcutaneous tissue, and superficial fascia may influence the sensory system.[1] Furthermore, skin and subcutaneous tissue ultrasonography (US) features may be altered by postural changes in healthy subjects and different patient conditions, such as lymphedema.[1,2]

Tensiomyography (TMG) appears as a new technological device for evaluating the contractile properties of skeletal muscles and has recently been applied to assess musculoskeletal conditions in the lumbar erector spinae muscles.[3] Nevertheless, skin thickness distributions, as well as the local hypodermal/subcutaneous fat, and fascia distributions may

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disturb the trunk dorsum sensitivity and should be investigated through future work.[1]

Therefore, the aim of this study was to describe and correlate the skin, subcutaneous tissue, and superficial fascia thickness assessed by US in the erector spinae muscles contractile properties evaluated by TMG.

MATERIAL AND METHODS

Study Design

A cross-sectional descriptive study was carried out between October 2015 and December 2016, following the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) guidelines and checklist.[4] Previously, the review board of the European University of Madrid (CIPI/039/15) had approved this study. Informed consent forms were signed by all participants before the beginning of the study. Furthermore, the Helsinki Declaration and ethical standards in human experimentation were considered. This research was funded and supported by the Real Madrid – European University (Ref.: 2015/09RM) and the European University of Madrid (Ref.:2015/UEM04).

Sample

A convenience sample of 50 healthy participants was recruited from the Faculty of Health, Exercise and Sport of the European University of Madrid. The inclusion criteria were healthy subjects, aged between 18 and 60 years, without bilateral non-specific pain as well as structural, neurological, visceral, or red flag conditions in the lumbopelvic region (between the subcostal line and the popliteal fossa).[3,5] The exclusion criteria were prior lumbopelvic pain or treatments (within the previous 6 weeks), or medical record of neuropathy, myopathy, rheumatoid arthritis, inability to follow instructions, cognitive impairments, dysmenorrhea, pregnancy, body mass index (BMI) greater than 31 kg/m^2, high-level athlete self-reported activity, skin disorders, conditions (such as fracture, structural deformities or neoplasm) and surgeries in the lumbopelvic or lower limb regions.[3,6]

Sociodemographic data

Gender, age (y), height (cm), weight (kg), occupation (teacher, administrative staff, sports monitor, or other occupations) and BMI (kg/cm^2) calculated by the Quetelet index were registered.[7]

Outcome measurements

Participants were placed in prone decubitus. Then, the point of maximum lordosis in the lumbar region of the right erector spinae (approximately 2 cm lateral to the 3rd lumbar vertebrae) was marked on the skin with a grid of 4 perpendicular lines. Furthermore, the outcome measurement order for each point was TMG and US in order to avoid the influence of the US gel temperature on the electrical stimulus.[3]

First, all US evaluations and measurements were performed by the same rater, who had over 4 years of experience. A diagnostic ultrasound system (Mindray Z6; Shenzhen Mindray Bio-Medical Electronics, Nansham, 518057, China) with a 5–10.0MHz range linear transducer (7 L4P type; 38-mm footprint), a frequency of 10.0MHz, a total depth imaging of 4cm and the focus located with a depth of 0.5cm were used to assess the resting B-mode US. The center of the probe coincided with the center of the skin marks (point of maximum lordosis) in a transversal and perpendicular position to the erector spinae muscle fibers. Skin (more superficial hyperechogenic band), subcutaneous tissue (hypoechogenic band under the skin), and erector spinae superficial fascia (hyperechogenic band under the subcutaneous tissue) US thicknesses measurements (cm) were performed in the center of the probe footprint with the software of the US system (Fig. 1). In addition, 3 ultrasound images were captured at the same point, at the end of expiration. The mean of the 3 repeated measurements was used for the data analysis. An excellent inter- and intraexaminer US reliability has been shown in the low back region.[1,3,6,8]

Second, TMG was used to assess the contractile properties of erector spinae muscles.[3] The five con-

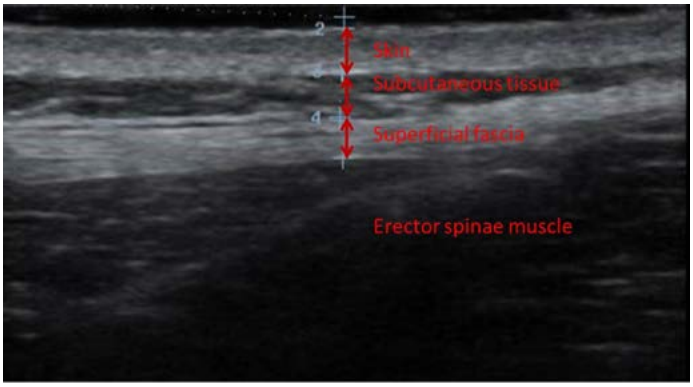

Fig. 1. US measurements of skin, subcutaneous tissue, and superficial fascia thickness. Abbreviations: US, ultrasonography.

tractile objective parameters were analyzed from the right erector spinae muscle belly displacement-time curves: maximal radial displacement (Dm; mm of displacement secondary to the muscle belly electrical stimulus), contraction time (Tc; ms from 10% to 90% of Dm in the ascending curve), sustain time (Ts; ms from 50% of Dm on both sides of the curve), delay time (Td; ms from the onset of electrical stimulus to 10% of Dm), and half-relaxation time (Tr; ms from 90% to 50% of Dm on the descending curve). Interexaminer reliability from good to excellent was stated for these contractile parameters.[9] The digital displacement transducer (GK 40, Panoptik d.o.o., Ljubljana, Slovenia) was placed perpendicular to the muscle belly on the point of maximum lordosis with an initial pressure of $1.5 \cdot 10^{-2}$ N·mm^{-2}, coinciding with the center of the skin marks.[10] Two circular self-adhesive electrodes (Model 3100C, Uni Patch, Wabasha, USA) with a diameter of 3.2cm were placed symmetrically at 1.6cm distal and proximal to the sensor tip (interelectrode distance of 3.2cm), longitudinally to the right erector spinae muscle belly (Fig. 2). A specialized researcher with over 4 years of TMG experience performed the measurements and data extraction. Finally, a TMG-S2 (EMF-FURLAN & Co. d.o.o., Ljubljana, Slovenia; 0-110 mA) stimulator was used to evaluate the erector spinae contractile properties at 100 mA of electrical current intensity during 1 ms (range from 0.5 to 2 ms) in order to avoid post-tetanic activation.[3,9]

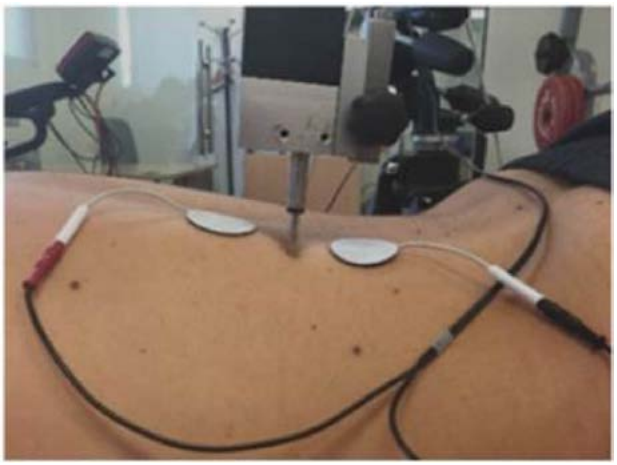

Fig. 2. TMG electrodes and digital displacement transducer placed perpendicular to the erector spinae muscle belly. Abbreviations: TMG, tensiomyography.

Statistical analysis

SPSS version 22.0 for Windows (SPSS IBM, Chicago, IL, USA) was utilized for the data analysis. First, Kolmogorov–Smirnov test was carried out to identify normal distribution (height, weight, BMI, Dm and skin thickness) or non-normal distribution (age, Td, Tc, Ts, Tr, subcutaneous tissue and superficial fascia thicknesses). Second, descriptive statistics were calculated depending on parametric (mean and standard deviation, SD) and non-parametric (median and interquartile range, IR) data. Finally, correlation analyses using Pearson (r for parametric data) and Spearman (r_s for non-parametric data) coefficients were performed to evaluate the relationship between the outcome measurements. Correlations were interpreted as weak (0.00–0.30), moderate (0.31–0.60), or strong (0.61–1.00).[3,11] A 95% confidence interval (P<0.05) was considered for all data analyses.

RESULTS

A sample of 50 participants, 29 (58%) men and 21 (42%) women, with an age median (IR) of 36 (11.50) years as well as height, weight and BMI mean (SD) of 172.94 (8.99) cm, 72.11 (15.05) kg and 23.91 (3.58) kg/cm^2, respectively, was recruited. Regarding the occupations, there were 33 (63%) teachers, 7 (14%) members of administrative staff, 5 (10%) sports monitors, and 5 (10%) with other occupations. US measurements showed a skin thickness

TABLE. CORRELATIONS BETWEEN ERECTOR SPINAE TMG CONTRACTILE PROPERTIES AND THICKNESS US MEASUREMENTS OF THE SKIN, SUBCUTANEOUS, SUPERFICIAL FASCIA AND TOTAL TISSUES.

TMG parameters (n = 50)	Skin thickness	Subcutaneous thickness	Fascia thickness
Dm	r = -0.329* (P = .020)	r_s = -0.668** (P < .001)	r_s = -0.252 (P = .077)
Td	r_s = 0.023 (P = .873)	r_s = 0.058 (P = .687)	r_s = -0.003 (P = .986)
Tc	r_s = -0.103 (P = .475)	r_s = -0.239 (P = 0.095)	r_s = 0.009 (P = 0.953)
Ts	r_s = -0.216 (P = .133)	r_s = -0.240 (P = .093)	r_s = -0.108 (P = 0.456)
Tr	r_s = -0.123 (P = 0.397)	r_s = -0.369** (P = 0.008)	r_s = -0.017 (P = 0.904)

Abbreviations: Dm, maximal radial displacement; r, Pearson correlation coefficient; r_s, Spearman correlation coefficient; Tc; contraction time; Ts, sustain time; Td, delay time; Tr, half-relaxation time; TMG, tensiomyography; US, ultrasonography. * P < .05 statistically significant correlations. ** P < .001 statistically significant correlations.

mean (SD) of 0.29 (0.04) cm as well as subcutaneous tissue and superficial fascia thickness medians (IR) of 0.30 (0.31) and 0.28 (0.12) cm, respectively. TMG measurements showed a Dm mean (SD) of 3.65 (1.98) mm as well as Td, Tc, Ts and Tr medians (IR) of 18.07 (2.80), 16.09 (3.42), 70.59 (322.34) and 39.93 (171.70) ms, respectively. As shown in the Table, a strong negative correlation was found between Dm and subcutaneous tissue thickness (r_s=-0.668; P<0.001). In addition, moderate negative correlations were observed between Dm and skin thickness (r=-0.329; P=0.020), as well as Tr and subcutaneous tissue thickness (r_s=-0.369; P=0.008). The other parameters did not show statistically significant correlations (P>0.05).

DISCUSSION

This novel study supports the use of US during Dm and Tr TMG parameter assessment in order to evaluate the relationship of skin and subcutaneous tissue thicknesses in the evaluation of lumbar erector spinae contractile properties. Furthermore, Dm has widely been used to assess muscle stiffness, and its strong negative correlation with subcutaneous tissue thickness may have altered the TMG response.[12,13] Therefore, prior TMG studies may have been influenced by the thickness of these tissues in the lumbopelvic region.[3] In addition, the spine postures between extension and flexion may alter the skin thickness from 12% to 38%. Consequently, such large structural deformations of the skin of the trunk dorsum should be considered in order to determine their influence in sensitivity assessments.[1]

Limitations

As limitations of the present study, Tr has shown insufficient reliability compared to the inter-rater reliability for the rest of TMG contractile parameters.[9] Furthermore, Dm may be modified depending on each muscle group, cross-sectional muscle area, and subject, according to the morphofunctional and training characteristics.[14] Finally, the small sample size and correlations in lumbopelvic conditions should be considered in future research.

CONCLUSIONS

The lumbar erector spinae contractile properties during TMG assessments, especially Dm and Tr, may be widely correlated with the skin and subcutaneous tissue thickness. Therefore, we encourage authors to consider these tissues during intersubject evaluations in future TMG research.

CONFLICT OF INTEREST AND SOURCE OF FUNDING STATEMENT

This research was funded and supported by the Real Madrid – European University (Ref.: 2015/09RM) and the European University of Madrid (Ref.:2015/UEM04).

RESUMO

CONTEXTO: *O estudo foi elaborado para descrever e correlacionar a pele, o tecido subcutâneo e a espessura da fáscia superficial avaliados pelo ultrassom (EUA) com as propriedades contráteis do músculo eretor da coluna lombar avaliadas por tensiomiografia (TMG).*

MÉTODOS: *Foi realizado um estudo descritivo transversal com 50 participantes saudáveis. O ponto de lordose máxima na região lombar da coluna ereta direita foi avaliado pelos EUA e TMG. Primeiro, a pele, o tecido subcutâneo e as espessuras da fáscia superficial (cm) foram avaliadas pelos EUA. Em segundo lugar, os cinco parâmetros TMG contráteis foram analisados a partir das curvas de deslocamento-tempo da barriga do músculo eretor da espinha direita: deslocamento radial máximo (Dm), tempo de contração (Tc), tempo de sustentação (Ts), tempo de atraso (Td) e meio tempo de relaxamento (Tr). Finalmente, foram realizadas análises de correlação usando os coeficientes Pearson (**r** para dados paramétricos) e Spearman (r_s para dados não paramétricos).*

RESULTADOS: *Uma correlação forte negativa foi mostrada entre Dm e espessura subcutânea do tecido (r_s = −0,668; **P** < 0,001). Além disso, foram observadas correlações moderadas negativas entre Dm e espessura da pele (**r** = −0,329; **P** = 0,020), bem como a espessura subcutânea do tecido (r_s = −0,369; **P** = 0,008). O restante dos parâmetros não mostrou correlações estatisticamente significativas (**P** > 0,05).*

CONCLUSÃO: *Portanto, as propriedades contráteis do eretor da espinha lombar durante as avaliações TMG, especialmente Dm e Tr, podem ser amplamente correlacionadas com a pele e a espessura subcutânea do tecido.*

PALAVRAS-CHAVE: *Contração muscular. Pele. Tela subcutânea. Ultrassonografia.*

ABREVIATURAS: *IMC: índice de massa corporal; Dm: deslocamento radial máximo;* **r:** *coeficiente de correlação de Pearson;* r_s*: coeficiente de correlação de Spearman; Tc: tempo de contração; Ts: tempo de sustentação; Td: tempo de atraso; Tr: meio tempo de relaxamento; TMG: tensiomiografia; US: ultrassonografia.*

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REVIEW ARTICLE

Platelets volume indexes and cardiovascular risk factors

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SUMMARY

Obesity, diabetes and hypertension are risk factors for cardiovascular diseases (CVD) because they promote a state of hypercoagulability. It is known that platelets play an important role in the development of atherosclerosis. Recent studies have evaluated platelet volume indexes (PVIs) in individuals with risk factors for CVD to better understand the platelet mechanisms involved in their development. The IVPs indirectly estimate platelet function and are easily obtained from automated hematology analyzers, which provide platelet counts, mean platelet volume (MPV), platelet distribution width (PDW) and the platelet-large cell ratio (P-LCR). The present study aims to review literature studies that investigated the association between PVIs and obesity, diabetes, and arterial hypertension, in order to evaluate its use as a potential subclinical marker of CVD. Studies have shown promising results for MPV, an index that allows for early detection of platelet activation and may be useful in identifying patients before the onset of CVD development so that preventive strategies can be implemented. The PDW, although evaluated by a smaller number of studies, also showed promising results. However, there is still a long way to go in order for the MPV and PDW to be used in clinical practice, since there is still a need for more epidemiological evidence, establishing reference values, and standardizing the way results are presented.

KEYWORDS: *Platelets indexes. Obesity. Diabetes mellitus. Hypertension..*

INTRODUCTION

Cardiovascular diseases (CVD) are represented by a set of diseases that affect blood vessels and the heart. The pathophysiological mechanisms depend on the developing disease[1]. The coronary artery disease, cerebrovascular accident (CVA), and peripheral arterial disease involve atherosclerosis, a multi-factor disease that promotes accumulation of lipids, inflammatory cells and fibrous elements that are deposited on the walls of arteries and are responsible for the formation of greasy plaques or striae, which usually cause them to become obstructed[2].

It is known that platelets play an important role in the development of atherosclerosis and thrombi[3]. The platelet volume indexes (PVIs) can directly estimate the platelet function and are easily obtained from more modern automated hematology analyzers[4]. The analyzers provide platelet counts, mean platelet volume (MPV), platelet distribution width (PDW), and platelet-large cell ratio (P-LCR). Larger platelets are metabolically and enzymatically more active in comparison to smaller ones because they contain more alpha granules, produce more throm-

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boxane A2 and feature high expression of adhesive glycoproteins[5].

Therefore, studies have investigated the association between PVIs and risk factors for CVD, among which are obesity, diabetes, and hypertension, as an attempt to better understand the platelet mechanisms involved in its development[1,3,6-9]. However, the results have been conflicting and despite the relative ease of obtaining these indexes, their use in clinical practice is still limited by the absence of standardization of procedures for measuring and interpreting its results. Thus, the presents study aims to review studies in the literature that investigated the link between PVIs and obesity, diabetes, and hypertension in order to assess its use as a potential subclinical marker of CVD.

PVI AND OBESITY

Obesity is a chronic metabolic disorder characterized by the excessive accumulation of body fat[10]. According to the World Health Organization (WHO), in order for an individual to be considered obese, they need to present a body mass index (BMI) equal or superior to 30kg/m² [11]. Obesity has been considered an epidemic and is a recognized risk factor for the development of CVD[12].

The platelet function can be modulated by metabolism and body weight regulators. The leptin hormone, for example, in heightened in obese individuals and, therefore, contributes to the platelet hyperreactivity[13]. In addition, the platelet activation plays an important role in the development of atherothrombosis, which is a result of the interaction between alterations typical of obesity, such as: insulin resistance, inflammation, oxidative stress, and endothelial dysfunction[13]. In view of this, PVIs have been studied as possible biomarkers for predicting the global risk of CVD in obese individuals[14-17].

Coban et al.[16], in a before and after interventional study on with 60 female subjects, observed a mean MPV significantly higher in the group of obese women, in comparison with the non-obese group (8.18 ± 1.09 vs. 8.01 ± 0.95 fL, p=0.004). In the group of obese women, there was a positive correlation between the MPV and BMI (r=0.43, p=0.017) and the reduction of MPV and weight loss (r=0.41, p=0.024).

Ozkan et al.[14] analyzed 108 children aged 6-16 years in a case-control study and found that obese children with nonalcoholic fatty liver disease have significantly higher MPV compared to non-obese children (7.44-6.93 fL, p<0.01). Coban et al.[15], in another case-control study on with 200 subjects, observed a MPV significantly higher in the group of obese individuals, in comparison with the non-obese group (10.3 ± 1.2 vs. 9.0 ± 0.8 fL, p<0.01). In the group of obese individuals, the authors found a positive correlation between MPV and BMI (p<0.05). The researchers also analyzed the platelet count but found no significant difference between the groups.

On the other hand, Montilla et al.[17], in a cross-sectional study with 307 male individuals, did not observe any significant difference in MPV values between the groups with abdominal obesity (waist circumference ≥ 94cm) and without it (waist circumference <94cm). However, they observed that those individuals who were in higher terciles of MPV had higher glycemic levels (5.7 ± 0.6 mmol/L *vs.* 5.99 ± 0.7 mmol/L, p<0.05) e lower platelet count (251 ± 53 /mm3 *vs.* 196 ± 36 /mm3, p<0=0.001) in comparison with individuals in lower terciles. They also noticed that the MPV showed a positive correlation, however weak, with prothrombin activity (r=0.130, p<0.05).

Therefore, considering that obesity is a classic risk factor for the development of CVD and that, according to studies, MPV seems to be associated to obesity, it is suggested that such index can be used as a marker for cardiovascular risk.

PVI AND DIABETES

Diabetes Mellitus (DM) is characterized by a group of metabolic disorders that have in common hyperglycemia as a result of defects in the action/secretion of insulin. The current classification of DM proposed by the World Health Organization (WHO) is based on the etiology of the disease and includes four classes: DM type 1, DM type 2, gestational DM, and other specific types of DM[18].

DM type 2 is a worldwide public health problem due to its high morbidity and mortality rates[18]. It is characterized by insulin resistance with gradual and progressive loss of pancreatic beta cell function, with insufficient insulin production and chronic hyperglycemia[19]. Hyperglycemia presents a harmful effect on blood vessels and the risk of mortality due to CVD is reportedly correlated with the concentration of glucose in the blood[20].

Studies have shown that individuals with DM have larger platelets that release more prothrombot-

ic factors in comparison with individuals that do not have DM[23,24]. Patients with type 2 DM have a higher risk of coagulation abnormalities and thromboembolic events[25,26]. Inflammation, oxidative stress, a reduction in the calcium metabolism and in nitric oxide bioavailability, an increase in phosphorylation and glycosylation of cell proteins are all factors responsible for the increase in platelet activation and release of prothrombotic agents[22]. Increased MPV values on individuals with DM in comparison with individuals without DM suggest a compensatory production of larger and more active platelets in the face of a higher activation, which might favor the development of CVD in those individuals. Thus, the use of PVIs as markers of platelet function can be promising to assist in the cardiovascular risk stratification for individuals with DM.

Han et al.[27], in a cohort study that proposed to assess the association between MPV and the development of cerebrovascular accident (CVA), as well as of peripheral arterial disease (PAD) in individuals with DM found that the group in the higher tercile of MPV (≥7.9 fL) presented medium risk of CVA and PAD significantly higher in comparison with the MPV group in the lower tercile (≤7.3 fL) (29.9% *vs.* 2.8%, log-rank: p<0.001).

Iyidir et al.[28], in a case-control study develop with pregnant women with (case) and without (control) gestational DM (GDM) found that in the third trimester of pregnancy, MPV was significantly higher among cases in comparison with the control (8.80 ± 1.0 *vs.* 8.10 ± 0.7 fL, p=0.002), even after adjustment for confounding factors. There was no significant correlation between glycated hemoglobin (HbA1c) and MPV in the group of pregnant women with GDM. Analysis by ROC curve showed that MPV ≤ 8.4 fL is able to predict GDM with a sensitivity of 63% and specificity of 66% (95% CI: 0.59- 9-0.84).

Demirtas et al.[22], in a cross-sectional study, demonstrated that the values of MPV and PDW were significantly higher [MPV: 9.20 fL (8.7-9.9) *vs.* 8.80 fL (8.3-9.3), p<0.001 and PDW: 16.60 fL (15.0-17.8) *vs.* 15.40 fL (14.2-16.5), p<0.001] among the patients with DM compared to those who did not have the disease. Among individuals with diabetes, those who had HbA1c ≥ 7% presented higher MPV (9.40 ± 0.9 vs. 8.90 ± 0.8 fL) and PDW (16.60 fL, 15.3-18 *vs.* 16.00 fL, 15.0-17.0) than those with HbA1c < 7%. A cross-sectional study conducted by Lee et al.[23] found a positive association (OR=2.10, p=0.012) between MPV values and the DM diagnosis, even after adjusting for confounding factors.

Ulutas et al.[24], also in a cross-sectional study, found significantly higher values of MPV in the group of subjects with DM (8.30 ± 1.3 fL) compared to the group of individuals without DM (7.10 ± 1.0 fL; p<0.001). Among the individuals with DM, the values of MPV were higher for those with HbA1c >7% (8.30 ± 1.3 fL) compared to those with HbA1c ≤7% (7.50 ± 1.1 fL; p = 0.039). MPV presented a positive correlation with HbA1c (r=0.39, p<0.001) and with plasma glucose (r=0.41, p<0.001), as well as with diabetes duration (r=0.22 ; p=0.02).

Finally, Shimodaira et al.[29], in a cross-sectional study, compared MPV values between subjects without DM and pre-diabetics subjects and observed a significantly higher value in the pre-diabetic group (p<0.001). Multiple linear regression analyses showed that MPV was independent and positively associated with fasting plasma glucose (p=0.020; p<0.001) not only in pre-diabetic individuals, but also in subjects with normal plasma glucose levels (β=0.006, p<0.05).

PVI AND HYPERTENSION

Arterial hypertension (AH) is a clinical condition characterized by the sustained elevation in pressure levels and is frequently associated with metabolic disorders and target-organ functional/structural alterations[30]. It causes changes in the vascular endothelium and platelets, as well as an increase in plasma levels of prothrombotic substances, which can be related to the development of CVD[31,32]. Studies have shown that PVIs are increased in patients with AH compared to those without the disease. In this sense, the use of these indexes seems useful for cardiovascular risk stratification and might contribute to preventive and treatment measures for CVD[33,34].

Yazici et al.[33], in a case-control study, evaluated individuals with pre-hypertension (systolic blood pressure: 120-139 mmHg or diastolic blood pressure: 80-89 mmHg) compared to healthy controls, and observed that the MPV values were higher in the first group in comparison to the second (10.41 ± 0.93 fL vs. 9.56 ± 1.04 fL, p<0.01). There was an association between elevated MPV and pre-hypertension, even after adjusting for confounding factors (OR=0.044, 95% CI: 0.003 ±-0.0724, p=0.029). In addition, the MPV was correlated with the systolic blood pressure, BMI, and

insulin resistance in the control group (r=0.41, p<0.02, r=0.37, p<0.04, r=0.35 , p<0.05, respectively).

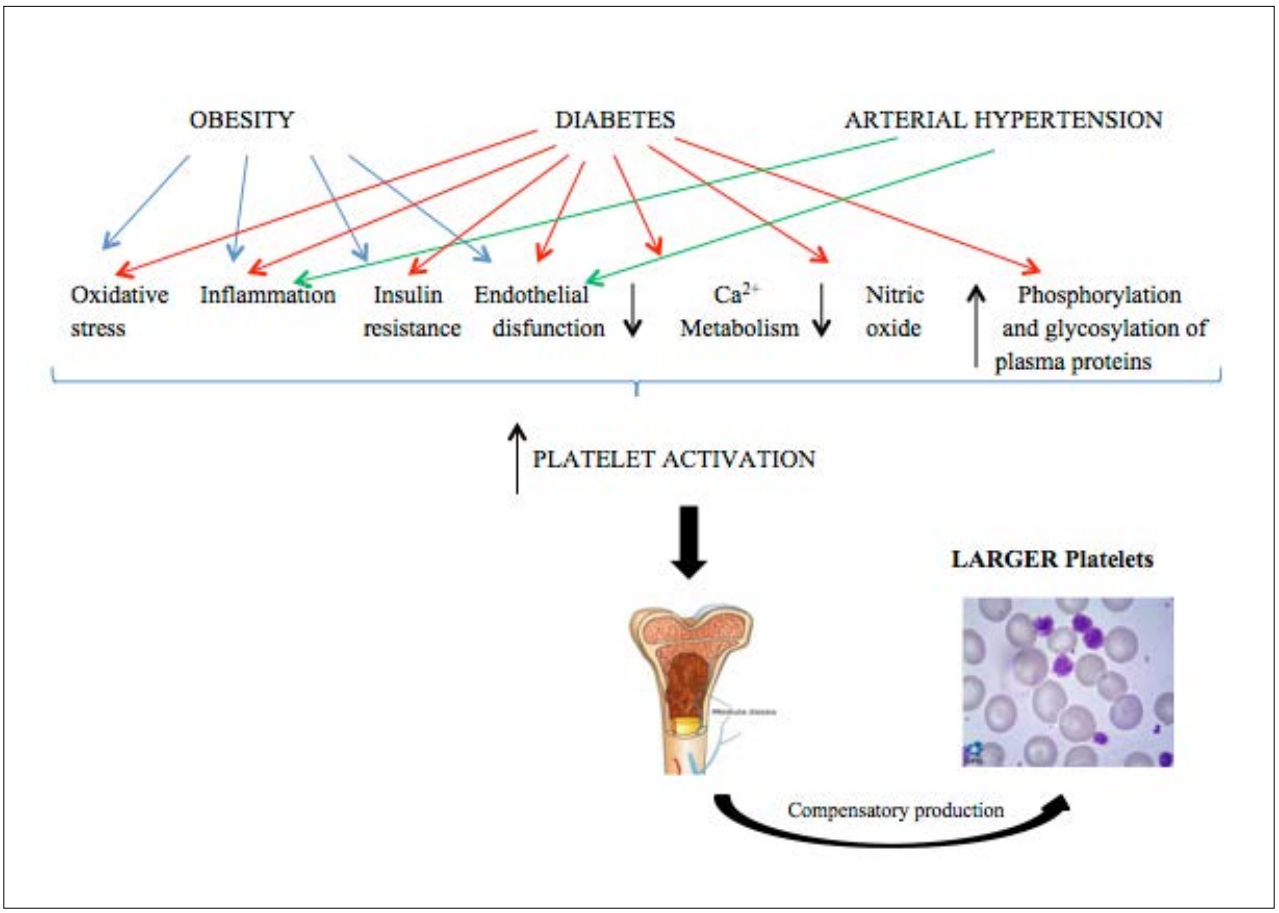
Coban et al.[34], in a case-control study, found that MPV was significantly higher in patients with essential hypertension and white coat syndrome than in normotensive individuals (p<0.00), whereas patients with essential hypertension showed higher MPV than those with white coat syndrome (p<0.05). The platelet count was not different between the groups studied (p>0.05) and MPV was positively correlated with the diastolic pressure in essential hypertension and white coat syndrome groups (p<0.05).

A cross-sectional study developed by Boos et al.[35] showed that patients with high-risk AH (HRAH) and malignant AH (MAH) had higher MPV than subjects without these diseases (7.80 ± 0.9 vs. 8.10 ± 1.0 *vs.* 7.40 ± 0.6 fL, p=0.0002, respectively). PDW was significantly lower among subjects with MAH (5.00 ± 0.5 vs. 5.40 ± 0.4 g/dL, p=0.001), with HRAH (5.00 ± 0.5 vs. 5.30 ± 0.5 g/dL, p=0.001) compared to individuals without these diseases. In the multivariate regression analysis performed with the hypertensive group (HRAH and MAH), a significant relationship was found between MPV and PDW (p<0.0001), systolic pressure (p=0.008) and platelet count (p<0.0001).

Yarlioglues et al.[36], in a cross-sectional study conducted only with men with arterial hypertension, found that MPV positively correlated with systolic and diastolic blood pressure (r=0.51, p<0.001 and r=0.55, p<0.001, respectively). Multiple linear regression analysis identified that levels of MPV were associated with proteinuria severity (coefficient =0.45, p=0.001), carotid intima-media thickness (coefficient =0.49, p=0.001), and ventricular mass increase (coefficient =0.48, p=0.001).

Erdogan et al. [37], also in a cross-sectional study, analyzed individuals with false uncontrolled AH, with white coat syndrome (group I), and individuals with true uncontrolled AH, with nocturnal reduction of systolic BP ≥ 10% (dipper) (group II), and with nocturnal reduction of systolic BP <10% (non-dipper) (group III). They observed that platelet counts were significantly lower in group III than in groups I and II (p<0.0001 and p<0.01, respectively) and was signifi-

FIGURE 1 - SCHEME OF BONE MARROW COMPENSATORY PRODUCTION OF LARGER PLATELETS IN INDIVIDUALS WITH OBESITY, DIABETES, AND/OR ARTERIAL HYPERTENSION.



cantly lower in group II than in group I ($p<0.0001$). MPV was significantly higher in group III than in groups I and II ($p<0.0001$ and $p<0.01$, respectively) and was significantly higher in group II than in group I ($p<0.0001$) .

AH is responsible for causing damage to the vascular endothelium, promoting platelet adhesion, activation, and aggregation, which causes a great peripheral consumption of platelets[31]. This process is likely the great responsible for inducing a compensatory production of enlarged platelets, which elevate MPV and PDW values in these individuals and consequently, increases the risk of CVD.

FINAL CONSIDERATIONS

The platelets and their pro-inflammatory and prothrombotic functions play an important role in the development of atherosclerotic plaques and thrombi formation. Thus, seeking to understand these mechanisms, many researchers have been focusing their interest in measuring platelet functions. The studies analyzed here showed that since the launch of the automated hematology analyzers, which release PVIs, MPV has been the most studied index, followed by PDW.

The great majority of studies has demonstrated an increased MPV in obese individuals with DM or AH in comparison with healthy individuals. People with those CVD risk factors present greater platelet activation, which promotes an increased peripheral consumption of platelets, with a consequent compensatory production of platelets that are larger and more reactive by the bone marrow[6].

A limitation of the analysis of the PVIs refers to the lack of standardization of the anticoagulants used in blood collection, since the use of sodium citrate is recommended for analysis or, in the case of EDTA collection, the analysis should be performed within two hours after collection, as it may cause an increase in platelet volume. In addition, another limitation in the analysis of the PVIs refers to the lack of reference values, which have not yet been established, and also the lack of standardization regarding the methodology used by the hematology analyzers, which can directly interfere in the comparability of the results.

However, even faced with these limitations, a considerable number of studies have demonstrated concordant and promising results for MPV, an index easily obtained by automated hematology analyzers with low costs. Thus, the early detection of platelet activation can be useful in identifying patients with increased thrombotic risk, before the beginning of CVD development, so that preventive strategies can be implemented.

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RESUMO

A obesidade, o diabetes e a hipertensão arterial são fatores de risco para as doenças cardiovasculares (DCV) por promoverem um estado de hipercoagulabilidade. É sabido que as plaquetas desempenham um importante papel no desenvolvimento da aterosclerose. Diante disso, estudos recentes têm avaliado os índices de volumes plaquetários (IVPs) em indivíduos com fatores de risco para DCV, para melhor se entenderem os mecanismos plaquetários envolvidos no seu desenvolvimento. Os IVPs estimam indiretamente a função plaquetária e são facilmente obtidos a partir de analisadores hematológicos automáticos, que fornecem contagens de plaquetas, volume médio de plaquetas (VPM), largura de distribuição de plaquetas (PDW) e a proporção de plaquetas grandes (P-LCR). O presente trabalho tem por objetivo revisar na literatura estudos que investigaram a associação entre os IVPs e obesidade, diabetes e hipertensão arterial, a fim de avaliar o seu uso como potencial marcador subclínico das DCV. Estudos demonstraram resultados promissores quanto ao VPM, um índice que permite uma detecção precoce da ativação de plaquetas e que pode ser útil na identificação de pacientes antes do início do desenvolvimento de DCV, de tal forma que estratégias preventivas possam ser implantadas. O PDW, embora tenha sido avaliado por um número menor de estudos, também demonstrou resultados promissores. Entretanto, ainda existe um longo caminho a se percorrer para que o VPM e o PDW sejam utilizados na prática clínica, pois ainda são necessárias mais evidências epidemiológicas, o estabelecimento de valores de referência e a padronização da forma de expressar os resultados.

PALAVRAS-CHAVE: *Índices de volume plaquetários. Obesidade. Diabetes* mellitus. *Hipertensão.*

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REVIEW ARTICLE

Isoflavones in gynecology

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SUMMARY

KEYWORDS: *Isoflavones. Menopause. Postmenopause. Hormone therapy.*

INTRODUCTION

Isoflavones are the most common forms of phytoestrogens and they are found in soy, soy products (soy milk, tofu, soy beverages, and soy flours), lentils, green peas, and alfalfa and bean sprouts. The main isoflavones are genistein, daidzein, and glycytine. They may be found in nonconjugate form (aglycone) and in conjugated form (glycosylated).[1] Isoflavones are nonsteroidal compounds structurally similar to natural estrogen, as they exhibit a phenolic ring with a hydroxyl radical attached to carbon three. This structure gives them a capacity for high-affinity selective binding to estrogen receptors, thereby enabling them to engage in estrogenic activity in human tissues. Isoflavones have an estrogenic or anti-estrogenic effect depending on their concentration, on endogenous sex steroids, and on the specific target organ in the interaction with the estrogen receptors. The fact that there are two types of estrogen receptors, alpha and beta, endows the different target organs with specificity to phytoestrogens.

ISOFLAVONE METABOLISM

Isoflavones are generally found in food in their main forms, as genistein, daidzein, and glycytine, i.e., bound to beta-glycosides and sugars. However, the human body does not absorb these forms. Thus, they change into smaller molecules through the action of specific enzymes for absorption without the sugar molecule.[2] Once ingested, the biologically inert glycosylated isoflavones undergo acid and enzymatic hydrolysis by gastric acids and intestinal glycosi-

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dases. However, it is the intestinal bacteria, through hydrolytic enzymes, that cause the changes in the glycosylated forms of isoflavones. The enzymes, which are called β-glucosidases, hydrolyze conjugated daidzein and genistein, converting them into their nonconjugate forms – namely aglycones– which can then be absorbed by the intestinal epithelium.[2] The bacteria break the heterocyclic bonds in the chemical structure of the isoflavone molecules changing these into phenolic acids, which can be absorbed, conjugated, excreted, or metabolized. The aglycone forms of isoflavone may undergo further transformation into other types of specific metabolites, such as equol or O-desmethylangolensin from daidzein, and p-ethynylphenol from genistein.[2]

Isoflavone absorption occurs by varied means and its metabolic pathways are not yet fully understood. After absorption, the molecules are believed to incorporate into chylomicrons, which in turn move into the lymphatic system and subsequently, into the circulatory system. By way of the latter, isoflavones reach all tissues, where they exert their biological effects, influencing the activity of all cells with estrogen receptors. After producing their estrogenic or anti-estrogenic effects, isoflavones enter the hepatic circulation, where they are secreted into bile, then partly reabsorbed into the enterohepatic circulation and partly excreted through feces and urine. The study of bacterial flora and of isoflavone metabolism is extremely important, for they have a bearing on blood concentration and on intestinal bioavailability and absorption.

ACTION MECHANISMS OF ISOFLAVONES

Isoflavone effects on the body vary from tissue to tissue given the affinity of isoflavones for specific receptors. The fact that isoflavones have a chemical structure similar to that of endogenous estrogens and that they bind to the same receptors as the latter, this enables isoflavones to regulate the gene expression of estrogen-regulated products.[3] There are two known types of estrogen receptors: estrogen receptor alpha (ERα) and estrogen receptor beta (ERβ). Isoflavones show greater affinity for ERβ, and estrogens for ERα.[3] The estrogenic potential of isoflavones is low compared to that of 17-β-estradiol, i.e., approximately 1/1000.[3] Genistein has a 4% binding affinity for ERα and 87% for ERβ, whereas daidzein, which is much less potent, exhibits a 1% and 5% affinity for ERα and ERβ, respectively. The high affinity of isoflavones for ERβ in comparison with ERα and the different distribution of such receptors in the tissues suggest a tissue-selective activity of the compounds. Hence, isoflavones would exert estrogenic action in some tissues, such as coronary vessels, but not in other tissues, such as the endometrium.[3,4] Some authors have classified isoflavones as selective modulators of estrogen receptors (SERMs). Isoflavones would act in similar ways to SERMs on estrogen receptors.[4] SERMs are nonsteroidal synthetic agents that bind to estrogen receptors inducing changes in the receptor's biological activity according to the type of tissue. Isoflavones may also exert their biological effects by means other than estrogen receptors. For example, isoflavones would act through tyrosine kinase receptors and other peptide receptors on the plasma membrane of certain cells. Other potential action mechanisms of isoflavones include cell-cycle regulation and antioxidant effects. Furthermore, isoflavones play an important role in preventing menopause-related disorders and chronic diseases, such as heart diseases, cancer, and diabetes.[5]

HORMONE THERAPY

Throughout a woman's life, ovarian changes take place leading to decreasing estrogen levels (hypoestrogenism) mainly associated with atrophy of the genital tract. Vulvovaginal symptoms develop and may include dryness, itching, burning, and pain, especially during sexual intercourse. Although the natural process of aging is a determinant of estrogen deficiency, it has been established that the chief etiological factors are vaginal epithelial deterioration and atrophic vaginitis.

To relieve hypoestrogenic symptoms, hormone therapy (HT) is currently the treatment of choice.[6] Vasomotor instability (hot flashes) frequently occurs in hypoestrogenism, and it manifests as a sudden sensation of intense heat along with flushing and sweating.[6] It usually lasts for less than 10 minutes. Vasomotor instability may also underlie anxiety symptoms, palpitations, and sleep disorders. Although the prevalence of vasomotor symptoms varies in accordance with race and ethnicity, over 50% of women report vasomotor symptoms at some point during menopause.

However, studies have attributed to estroprogestative or estrogen therapy the onset of side effects,

such as increased risk of breast and endometrial cancers and of thromboembolism. Therefore, its use has been questioned.[7] For this reason and given the increasing demand for alternative natural therapies, a significant number of women have been using phytoestrogens, particularly isoflavones, to relieve the symptoms of hypoestrogenism.[2]

ISOFLAVONES AS HORMONE THERAPY

Questions still linger about the beneficial effects of isoflavones on the female reproductive system and its appendages, particularly the breasts. Therefore, there is a demand for evidence-based benefits of isoflavone consumption in preventing and treating the undesirable effects of hypoestrogenism. The requirement is being met through experimental and epidemiological studies.

A prospective cohort study showed that the higher the isoflavone ingestion, the higher the protection against breast cancer in Latin, African, Japanese, and American women.[8] However, a study analyzing the effects of isoflavones on breast cancer-related genetic pathways demonstrated that a high concentration of plasma genistein induces the overexpression of genes that stimulate the cell cycle proliferation pathway. This raises the concern that the consumption of high soy concentrations could make women more vulnerable to breast cancer.[9]

In vitro studies report that high concentrations of isoflavones reduce cell proliferation, whereas low concentrations exert stimulating effects.[1] Low isoflavone concentrations are also capable of modifying the expression of some vital genes for cell survival, cell cycle control, and apoptosis.[10]

Laboratory studies have shown that genistein and daidzein can inhibit tyrosine kinase (PTK) by blocking the signaling pathway between the growth factor and its receptor and the DNA, thus thwarting activation of both cell proliferation and angiogenesis.[11] It is well known that kinase-dependent cyclins (KDCs) and cyclin-dependent kinase inhibitors regulate different phases of the cell cycle. Hence, these regulators are important targets for cancer therapy and prevention. In a study evaluating the combination of genistein and daidzein, the authors detected an increase in p53 and a reduction in cyclin B1 protein expression.[12]

Experimental work carried out with female rats receiving different isoflavone doses showed that isoflavones do not stimulate breast proliferation. Instead, they have a protective effect due to the reduced capacity of isoflavones to bind to ERα.[13] In a later study of the effect of soybean isoflavones on the expression of genes which control cell growth, the authors observed cell cycle blockage and potential cancer prevention.[14] Notwithstanding the numerous studies for or against the use of isoflavones in postmenopausal women with breast cancer, we believe they should not be indicated, for the studies are much too controversial.

In a case-control study of estrogen-dependent ovarian cancer conducted in Southern China, Lee et al. [15] reported that daily consumption of at least 120g of isoflavones, when compared to less than 61g, had a protective effect on women. They assumed the outcome was related to apoptosis induction and to growth and proliferation inhibition of the tumor cells. They added that isoflavones could boost the production of sex hormone-binding globulin (SHBG) in the liver, leading to a reduction in bioavailable estrogens in the plasma. Another hypothesized mechanism was the inhibition of aromatase activity in the ovary. This enzyme converts androgens into estrogens, and such is the case *in vitro*.

The examination of meta-analyses of randomized controlled trials to evaluate the effectiveness of phytoestrogens in vasomotor symptoms and their side effects in postmenopausal women revealed considerable divergence among authors. Nevertheless, most reported mitigation of the symptoms, as well as improvement in the quality of life; none reported any side effects.[16] On the other hand, Del Giorno et al.[17] showed there was no significant improvement in menopausal symptoms and sexual satisfaction after the use of isoflavones derived from *Trifolium pratenses*. There are many published meta-analysis studies of soy isoflavones and vasomotor symptoms. The most recent comprehensive meta-analysis examined the results of 19 clinical trials of soy isoflavones for treating hot flashes and concluded that isoflavone supplements derived from the chemical synthesis of plant extracts were significantly more effective than placebo in reducing the severity of the heat waves et al.[18]

Atrophy of the genitals in menopause ranges from 10% to 50%. The lack of circulating estrogens favors a reduction in collagen and elasticity, resulting in vulvovaginal atrophy and dryness. These in turn give rise to diminished lubrication, which causes discomfort and dyspareunia (pain) during sexual inter-

course.[19] Studies assessing isoflavone action on the vagina after menopause are scarce. [20] Epidemiological studies of postmenopausal women using gel isoflavone reported improvement in vaginal trophism with attendant improvement in vaginal symptoms, pH, and increase in estrogen receptor expression, indicating that isoflavones are possibly a good therapy option for vulvovaginal atrophy relief.[21] Experimental studies with female rats under long-term isoflavone treatment showed vaginal epithelium trophism, confirming an isoflavone-induced trophic effect.[13, 22]

A randomized double-blind study of postmenopausal women who consumed soy isoflavones showed that long-term use neither affects endometrial thickness nor increases hyperplasia or endometrial cancer.[23]

Studies conducted with female rats showed that genistein and daidzein induced several genomic responses in the uterus. However, dosages deemed normal did not stimulate cell proliferation and thus these isoflavones may be considered agonists and/or SERMs.[22] High dosages prompted isoflavones to have a trophic effect on the endometrium, but when combined with estrogens, they did not present an additive effect.[14] A prospective clinical study that assessed the endometrium of 32 menopausal women for six months stated that three women exhibited endometrial changes suggestive of endometrial stimulation.[24] In still another study involving high doses of isoflavones, the uterus of oophorectomized female rats presented endometrial squamous metaplasia.[13]

Asian countries have a lower fracture rate than Western countries, such as the United States. This difference may be related to the fact that soybean food products are rich in isoflavones and are consumed daily by Asian women. Thus, many studies report the beneficial effects of isoflavones as inhibitors of the effects of bone resorption. In this respect, they are similar to estrogen, which is known to suppress bone resorption activity. According to in vitro models, isoflavones suppressed osteoclast formation. A meta-analysis study reported that isoflavones significantly attenuated bone loss in postmenopausal women. [18]

The beneficial effects of isoflavones include not only a reduction in bone loss, but also the stimulus for bone formation and for increased bone mineral density.

Antioxidant properties of isoflavones in the female reproductive system

Isoflavones are also known for their antioxidant properties, among which the capacity for regulating the enzyme expression and activity of the antioxidant system and for inhibiting oxidation of cell components through direct sequestration of free radicals by its phenolic rings or its ability to chelate the metallic ions involved in the oxidative process.[25]

Excessive production of free radicals may create an inadequate environment for normal physiological reactions, giving rise to a number of diseases of the female reproductive system, including endometriosis, polycystic ovary syndrome (PCOS), and infertility, without any apparent cause.

In the female reproductive system, free radicals play a key role in the regulation of several signaling pathways in folliculogenesis and oocyte maturation, in the cyclic changes in the endometrium, and in embryo implantation. Hence, oxidative stress exerts its influence throughout a woman's reproductive lifespan and modulates the decline of fertility as a woman ages.[26]

A study conducted with infertile women showed that dietary soybean consumption while they underwent treatment with an assisted reproduction technique seemed positive for the likelihood of pregnancy.[27] Likewise, Unfer et al.[28] noted that the pregnancy to delivery rate among the women who underwent in vitro fertilization and received isoflavone supplementation was almost double the number of women who did not ingest any isoflavones. Another study with infertile Japanese women revealed that diets rich in genistein and daidzein could reduce the risk of deep endometriosis. [29]

Reduction in oxidative stress by isoflavones has been demonstrated in several in vivo models. Genistein and daidzein have also been associated with a decrease in the risk of chronic pathologies, such as neurodegenerative, cardiovascular, and metabolic diseases, as well as cancers, partly due to their antioxidant activities.[30]

An experimental study with sexually mature female rats revealed that treatment with genistein diminished follicular atresia and raised the number of surviving ovarian follicles, suggesting genistein contributes towards lengthening the reproductive lifespan.[31]

Finding that isoflavones play a role as antioxidants has widened its potential uses not only in treating hypoestrogenism-derived disorders, but also in preventing and treating conditions associated with an increase in oxidative stress.

CONCLUSIONS

Isoflavones play many roles in offsetting diverse menopausal symptoms. Nonetheless, further studies are required to ensure outcome reliability.

CONFLICT OF INTEREST

The authors declare no conflict of interest in relation with this paper.

PALAVRAS-CHAVE: *Isoflavonas. Menopausa. Pós-menopausa. Terapia hormonal.*

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