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Postal Code: 01333-903

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# Do phosphodiesterase-5 inhibitors have a cardioprotective effect?

 Fernando Nestor Facio Junior<sup>1</sup>  
 Ana Flávia Azevedo Querichelli<sup>2</sup>  
 Bruno dos Reis Santos<sup>3</sup>  
 Germano José Ferraz de Arruda<sup>4</sup>  
 Luís Cesar Fava Spessoto<sup>1</sup>

**1.** Professor de Urologia, Faculdade de Medicina de São José do Rio Preto (FAMERP / FUNFARME), São José do Rio Preto, SP, Brasil.

**2.** Médica, Clínica Médica; São Paulo, SP, Brasil.

**3.** Estudante Universitário, Faculdade de Medicina de São José do Rio Preto (FAMERP), São José do Rio Preto, SP, Brasil.

**4.** Urologista, Faculdade de Medicina de São José do Rio Preto (FAMERP / FUNFARME), São José do Rio Preto, SP, Brasil.

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Erectile dysfunction (ED) affects 18 million men in the United States alone<sup>1</sup>. Its prevalence is 52% among men aged from 40 to 70 years and 70% among those older than 70<sup>2</sup>. This condition may be related to changes in penile blood flow since the etiology is vascular in 60% of patients<sup>1</sup>.

Cardiovascular disease (CVD) and ED share the same risk factors, such as age, smoking, hypertension, obesity, diabetes mellitus, sedentary lifestyle, depression, and metabolic syndrome<sup>1,3</sup>. Such factors lead to endothelial dysfunction, the formation of atherosclerotic plaque, and the inflammation of small blood vessels and are, therefore, related to both cardiovascular events and ED. The evaluation of the temporal relation between ED and subclinical CVD may encourage a change in risk behaviors and prevent the occurrence of CVD<sup>4</sup>. Studies have demonstrated that patients with ED have an increased risk for cardiovascular events and mortality and that ED may be a symptom or even an independent predictor of coronary artery disease (CAD)<sup>1,2</sup>.

Arterial hypertension is related to the development of atherosclerosis and is one of the risk factors for the development of CAD and ED but is also a protective factor against ED in patients with CAD, as it improves penile function<sup>1</sup>. In ED, the increase in vascular pressure initially improves penile flow and perfusion but is harmful in the long term since it implies an increase in atherosclerosis and arterial occlusion<sup>1</sup>. In urology, the pharmacological treatment of ED is based on the use of vasodilators, especially the class of phosphodiesterase-5 (PDE-5) inhibitors, which improve blood flow and sexual performance (sildenafil, vardenafil, tadalafil, and avanafil)<sup>5</sup>.

PDE-5 inhibitors diminish the catabolism of cyclic guanosine monophosphate (cGMP) through the inhibition of the enzyme PDE-5, which results in the relaxation of smooth muscles and consequent vasodilation, besides offering a cardioprotective effect related to the action of nitric oxide<sup>5</sup>. The discovery that PDE-5 is also expressed in the myocardium led to a change in views regarding the direct effect of these inhibitors

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CORRESPONDING AUTHOR: Fernando Facio Jr.

Av. Fernando Correa Pires, 3600, São José do Rio Preto, SP, Brasil – 15015-040

E-mail: fnfacio@yahoo.com.br

on this tissue<sup>6</sup>. The expression of PDE-5 suggests that inhibitors of this enzyme exert beneficial effects on the heart through changes in regional and systemic hemodynamics related to anti-apoptotic and anti-inflammatory properties as well as reductions in hypertrophy, ventricular arrhythmia, myocardial fibrosis, and oxidative stress<sup>5</sup>.

There is evidence of the effectiveness of PDE-5 inhibitors in heart failure with reduced ejection fraction. Such evidence is sustained by the effects on pulmonary vasodilation, cardiac remodeling, improved diastolic function, reductions in fibrosis and interstitial inflammation, and an improvement in coronary microcirculation<sup>6</sup>. Regarding myocardial infarction, the use of PDE-5 inhibitors seems to increase the tolerance threshold for ischemia/angina and is related to a lower mortality rate after the event<sup>6</sup>. For pulmonary hypertension, treatment with PDE-5 inhibitors is effective due to vasodilation, a reduction in cell proliferation, and the stimulation of apoptosis, which have the potential to reduce pulmonary pressure<sup>7</sup>.

In a retrospective study conducted in Denmark, patients in treatment with PDE-5 inhibitors for ED with no history of cardiovascular disease had a lower

risk of acute myocardial infarction in comparison to the general male population<sup>3</sup>. Moreover, the risk of heart failure and all cardiovascular diseases diminished in the first three years after the start of treatment for ED<sup>3</sup>. This effect may be explained by the pharmacological action of PDE-5 inhibitors through the reduction of systolic blood pressure after the beginning of treatment, the long-term cardioprotective effect, or factors associated with behavioral changes in patients<sup>3</sup>.

Since the recognition of this vasodilating effect, the interest in employing PDE-5 inhibitors for the treatment of CVD has increased and these drugs are currently used in patients with ischemic heart disease, congestive heart failure, pulmonary hypertension, Raynaud's phenomenon, peripheral vascular disease, and cerebral hypoperfusion<sup>7</sup>. Further prospective, randomized, clinical investigations are needed to explore the therapeutic potential of PDE-5 inhibitors as well as determine the effectiveness and safety of these drugs with regards to the cardioprotective effect.

### Conflicts of Interest

The authors have no conflicts of interest to declare.

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# Upper urinary tract obstruction during the pandemic: what should be done?

 Germano Arruda<sup>1</sup>  
 Jerônimo Arruda Neto<sup>2</sup>  
 Luis Cesar Spessoto<sup>3</sup>  
 José Germano Arruda<sup>3</sup>  
 Fernando Fácio Jr<sup>3</sup>

1. Mestrando do Programa de Ciências da Saúde, Faculdade de Medicina (FAMERP), São José do Rio Preto, SP, Brasil.

2. Residente de Cirurgia Geral, Hospital São Domingos. Catanduva, SP, Brasil.

3. Professor de Urologia, Departamento de Urologia. Faculdade de Medicina (FAMERP/FUNFARME), São José do Rio Preto, SP, Brasil.

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The new coronavirus (SARS-CoV-2) has quickly become a tragic medical emergency throughout the world. Despite national policies and the efforts of health authorities to contain the virus, the growing number of cases and deaths in our country has led to concerns regarding the collapse of the healthcare system, as it is necessary to prioritize an increasing number of infected individuals while maintaining the treatment of all non-delayable and oncological cases.<sup>1</sup>

Urinary calculus is an urgent condition with a high potential for complications and deterioration of kidney function.<sup>2</sup> Therefore, the timely management of this condition is crucial. Treatment, which ranges from mildly invasive procedures with local anesthesia to major surgeries under general anesthesia, requiring muscle relaxants and anesthetic sprays, is well established and has precise indications. However, COVID-19 has altered the conduct for surgical patients, leading to the complete suspension of services in some cases since the onset of the pandemic. Based on limited experience, national and international institutions have begun to issue guidelines for the management

of such patients. To date, however, there are no safe, well-established guidelines for surgical practice during the pandemic.<sup>3</sup>

The consensus is that elective procedures for patients with kidney stones, few symptoms and no complications of the upper urinary tract can be postponed for up to six months without compromising kidney function.<sup>4</sup> Partially obstructive stones should be evaluated individually with the calculation of the risk-benefit ratio of surgical intervention during the pandemic and discussing the options with the patient since complications are possible if treatment is delayed for three or four months.<sup>4</sup>

For patients already admitted to the emergency ward with refractory colic, acute kidney failure, or acute pyelonephritis, which are situations with a high potential for morbidity and mortality, an intervention within a short period of time is fundamental, regardless of the pandemic.<sup>5</sup> In such cases, the clearance of the urinary system should be performed urgently, even considering the scarcity of anesthetics. The clinical severity of these cases merits additional attention,

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CORRESPONDING AUTHOR: Germano José Ferraz de Arruda

Departamento de Urologia, Faculdade de Medicina de São José do Rio Preto (FAMERP)

Av. Brigadeiro Faria Lima, 5416, Vila São Pedro, São José Rio Preto, SP, Brasil

Tel: +55 17 99602-2914

E-mail: germano\_lito@yahoo.com.br



as the time spent hospitalized and perhaps in the intensive care unit can cause greater morbidity and mortality as well as an excessive financial cost. Screening for SARS-CoV-2 infection in patients admitted with obstructive urolithiasis should be performed in accordance with established local and regional guidelines.<sup>4</sup>

The Brazilian Society of Urology recognizes the complexity of the current scenario and the unfeasibility of strict rules with regards to undergoing or postponing a surgical procedure, which can frustrate expectations, affect the course of the disease, and generate insecurity in patients. Decisions should always

involve the medical opinion and the participation of the patient and/or family members.<sup>6</sup>

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The authors of this manuscript have no conflicts of interest to disclose.

### Author's Contribution

All authors have contributed equally to this work

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# Use of daratumumab in patients with amyloidosis

## Participants:

Adriano Anza<sup>1</sup>  
 Armelin Utino<sup>1</sup>  
 Haroldo Katayama<sup>1</sup>  
 Ighor A. Z. Spir<sup>1</sup>  
 Marcio A. Lemos<sup>1</sup>  
 Mauricio Anhesini<sup>1</sup>  
 Oswaldo S. Tiezzi<sup>1</sup>  
 Patricia R. N. Spir<sup>1</sup>  
 Pericles Otani<sup>1</sup>  
 Wanderley M. Bernardo<sup>2</sup>

1. Grupo de M.B. E – Presidente Prudente, Presidente Prudente, SP, Brasil

2. Coordenador do Programa Diretrizes da Associação Médica Brasileira, São Paulo, SP, Brasil

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Contact: wmbernardo@usp.br

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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.*

## INTRODUCTION

The use of Daratumumab for treating amyloidosis is currently considered ‘off label’.

Amyloidosis (AL), of light chain (LC) immunoglobulin, is a protein deposition disease characterized by different degrees of organic dysfunction, morbidity, and early death due to LC toxicity, usually produced by a population of clonal plasma cells. Cardiac involvement is common, and patients with severe heart failure have a poor prognosis, with a mean survival of 6 months and 40% in 1 year. Successful LC suppressive therapy, measured by a criterion of hematogenic response, is associated with better rates of organic response and survival. Although initial LC suppressive therapy provides high rates of overall hematologic response, most patients do not reach a complete hematological response to the treatment,

allowing for the evolution of toxic amyloid deposition. Improvements in the organic functions involved are even less common. In addition, the underlying dyscrasia of plasma cells is not curable, and almost all patients eventually relapse, requiring additional LC suppressive therapy.

Treatment regimens for AL include proteasome inhibitors, alkylating agents, and steroids, as well as autologous stem cell transplant (HSCT) autologous in young patients.

Daratumumab is a G1k (IgG1k) monoclonal antibody of human immunoglobulin that targets the surface antigen CD38 in plasma cells and has demonstrated efficacy in multiple myeloma. Although the biology of clonal plasma cells in AL is distinct from that in multiple myeloma (lower cell proliferation),



they express CD38, providing then a proposal for an extrapolated use of Daratumumab in Amyloidosis.

## CLINICAL QUESTION

Does the use of Daratumumab in patients with Amyloidosis bring benefits in comparison to the conventional treatment?

**PICO (P: population, I: intervention; C: comparison; O: outcome)**

P: Patients with Amyloidosis

I: Daratumumab

C: conventional treatment

O: clinical improvement

## ELIGIBILITY CRITERIA

PICO components

No restrictions to study design

No time or language restrictions

Full texts available

## DATABASES SEARCHED

Medline (via Pubmed) and Cochrane Central

## SEARCH STRATEGY

(amyloidosis AND daratumumab)

## RESULTS

Were retrieved 59 works by combining the terms Amyloidosis and Daratumumab. Of these, we selected 8 studies that met the eligibility criteria. There is no randomized clinical trial completed up until now

## SELECTED EVIDENCE<sup>1-8</sup>

**Gran C 2017<sup>1</sup>**

Report of 2 cases of severe AL, one with severe heart failure and another with heart and kidney failure, undergoing treatment with Daratumumab. Both patients showed a rapid decrease in free light chains (FLC) in response to infusions of Daratumumab, with few associated adverse events. Very high risk of bias.

**Kaufman GP 2017<sup>2</sup>**

A retrospective analysis of patients with AL

(biopsy and immunohistochemistry or mass spectroscopy) treated with intravenous Daratumumab (16mg/kg/week) for 8 weeks, followed by 8 doses in alternate weeks, and then every 4 weeks. The doses were administered for 8 hours with 500ml of intravenous fluids, with the exception of the first dose, which was divided in half and administered in 2 consecutive days. If well tolerated, the doses could be administered for a minimum of 4 hours. All patients received premedication with acetaminophen (650 mg), diphenhydramine (50 mg), and dexamethasone (20 mg). After the initial infusion, dexamethasone was reduced per medical criterion. Twenty-five patients received a median of 12 (3-35) infusions of Daratumumab. All patients were evaluated for toxicity and 24 were evaluated for hematological response. The overall rate of hematological response was 76%, including complete response (CR) in 36% and partial response in 24%. The median response time was 1 month. Infusion reactions of grade 1-2 occurred in 15 patients, but no reactions of grade 3 or 4 were observed. Very high risk of bias.

**Sher T 2016<sup>3</sup>**

Report of 2 cases of AL, one with liver and heart failure and another with kidney failure, undergoing treatment with Daratumumab. Both patients showed a rapid decrease in free light chains (FLC) with adverse events that did not lead to the loss of adhesion. Very high risk of bias.

**Despande S 2020<sup>4</sup>**

A case report of a patient with involvement of multiple organs associated with nerve impairment; due to the nerve involvement, Daratumumab was chosen as the first drug, receiving the standard dose of 16 mg/kg in 8 weekly infusions for 8 weeks (8 cycles) in association with dexamethasone 20mg before the Daratumumab infusion. There was no infection, reduction in serum M-peak and serum lambda free light chain, improvement of neuropathy, with a decrease of gabapentin, and improvement of liver and kidney functions

**Chung A 2019<sup>5</sup>**

A cohort study of 72 patients with a mean age of 67 years, previously treated for AL amyloidosis, included in the study between January 2016 and January 2019.

The patients received Daratumumab at a dose of 16 mg/kg/week for 8 weeks, and 8 additional doses every two weeks and then every 4 weeks, with

dexamethasone (20 mg), and were evaluated retrospectively. The mean follow-up time was 27 months.

A total of 40 of the 52 evaluated patients achieved hematological response (77%) in an average time of one month; 57 patients had cardiac involvement, and 55% of them had a cardiac response in an average time of 3.2 months; 47 patients had renal involvement, and 52% of those evaluated had a renal response in an average time of 6 months.

The rate of overall survival in 2 years was 86%.

#### Lecumberri R 2020<sup>6</sup>

A retrospective multicenter study on the treatment of relapsed or resistant evaluated 38 patients, 76% with cardiac involvement, 74% with renal involvement, and 42 % with the involvement of  $\geq 3$  organs. The average number of previous therapies was 02. Hematological response in 72%, including 28% of complete response. The cardiac response was 37% e kidney 59%. The overall progression-free survival was 59% for the heart and 52% for the kidney.

#### Schwotzer R 2019<sup>7</sup>

A multicenter observational study of 10 patients, included in the study between August 2017 and October 2018, mean age 62.3 years, with LC Amyloidosis previously treated (resistant or relapsed) and a load of plasma cells from the bone marrow equal to or above 10%, who feature as the patients with worse outcomes when compared to those below 10%; all patients had cardiac involvement.

Daratumumab was administered at a dose of 16 mg/kg/week for 8 weeks, followed by 8 additional doses every two weeks and a maintenance dose every 4 weeks, plus 20 to 40 mg of dexamethasone.

The overall hematological response was 90%, with 70% of high quality, reaching this response after 73 days, on average; 50% of the patients presented cardiac response in 3.8 months, on average.

After an average follow-up time of 10 months, 8 patients were still receiving medication.

#### Sanchowala V 2020<sup>8</sup>

A prospective phase 2 trial of monotherapy with Daratumumab was designed to determine its safety, tolerability, and clinical and hematological responses.

22 patients had already been formerly treated for amyloidosis. There were no relevant adverse reactions during the infusion. The adverse reactions observed were respiratory infections (4 patients) and atrial fibrillation (4 patients). There was a complete hematological response and partial very good response in 86%. Renal response occurred in 10 of 15 patients with renal involvement; 7 of 14 patients with cardiac involvement obtained a response. The average follow-up of the surviving patients was 20 months.

### QUALITY OF EVIDENCE<sup>9</sup>

The evidence selected has a very high risk of bias (3 historical cohort studies, 2 case series, and 3 case reports), which in itself determines a very low strength. Thus, the level of scientific uncertainty is too high to ensure that the effects are produced by the intervention being tested; thus, future consistent evidence is necessary, which may even demonstrate damages and not benefits from the intervention.

Another 3 works were selected for resistant treatment/recurrence of amyloidosis using Daratumumab, two of them retrospective and one in phase 2 of the study, with very low strength of evidence.

### SYNTHESIS OF EVIDENCE

The use of Daratumumab in patients with clinical symptoms of Amyloidosis currently should not be recommended due to the weakness of the evidence available to support it.

In patients with resistant or recurrent Amyloidosis, observational studies have demonstrated good results with the use of Daratumumab associated with Dexamethasone.

### PERSPECTIVES

Currently, there are 8 clinical trials registered on ClinicalTrials.gov studying the use of Daratumumab for the treatment of patients with Amyloidosis<sup>10</sup>. Among these, there is a randomized clinical trial with 416 participants comparing the drug with the standard treatment, which should be completed in 2024<sup>11</sup>.

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# Ultrasound in the first trimester of pregnancy

 Glauce Romeiro de Almeida<sup>1</sup>

 Ricardo dos Santos Simões<sup>1</sup>

 Antonio Silvinato<sup>1</sup>

 Wanderley Marques Bernardo<sup>2</sup>

Created on: November 2020

E-mail: [wmbernardo@usp.br](mailto:wmbernardo@usp.br)

1. Programa Diretrizes da Associação Médica Brasileira, São Paulo, SP, Brasil.

2. Coordenador do Programa Diretrizes da Associação Médica Brasileira, São Paulo, SP, Brasil.

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**QUESTION: What is the goal of first-trimester ultrasound?<sup>1</sup>**

**Answer:** There is no reason to perform it as a routine exam, just to confirm pregnancy, in the absence of any risk factor.

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# The occurrence of recently diagnosed atrial fibrillation in the immediate postoperative period after myocardial revascularization surgery: although common, an underrated complication

 Marcel de Paula Pereira<sup>1</sup>  
 Eduardo Gomes Lima<sup>1</sup>  
 Cibeles Larrosa Garzillo<sup>1</sup>  
 Camila Talita Machado Barbosa<sup>1</sup>  
 Leon Pablo Cartaxo Sampaio<sup>1</sup>  
 Francisco Carlos da Costa Darrieux<sup>2</sup>  
 Carlos Vicente Serrano Jr<sup>1</sup>

**1.** Unidade de Aterosclerose – Instituto do Coração (Incor) do Hospital das Clínicas da Faculdade de Medicina da Universidade de São Paulo (HCFMUSP), São Paulo, SP, Brasil  
**2.** Unidade de Arritmia – Instituto do Coração (Incor) do Hospital das Clínicas da Faculdade de Medicina da Universidade de São Paulo (HCFMUSP), São Paulo, SP, Brasil

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## SUMMARY

Atrial fibrillation (AF) is the most common type of arrhythmia in the postoperative period after cardiac surgery, with a prevalence of 15-40% after myocardial revascularization surgery. Various strategies have been tested to prevent and manage AF in the postoperative period. Previous studies and record analyses have already demonstrated higher rates of hospitalization and clinical outcomes associated with this condition, including increased mortality in the short- and long-term. This point of view brings a review of the subject, with the most updated recommendations for managing this arrhythmia in the postoperative period after myocardial revascularization surgery, focusing particularly on anticoagulation strategies.

**KEYWORDS:** Cardiac Surgical Procedures. Myocardial Revascularization. Coronary Artery Bypass. Atrial Fibrillation. Anticoagulants.

Atrial fibrillation (AF) is the most common type of arrhythmia in clinical practice. Its occurrence is common in the postoperative period after cardiac surgery, with a prevalence of 15-40% after myocardial revascularization surgeries (MRS), it is the most common complication from this surgery<sup>1-6</sup>. The peak incidence occurs around the 2<sup>nd</sup> postoperative day. The

pathogenesis is multifactorial and probably involves the adrenergic stress related to the surgical procedure, inflammation, myocardial ischemia (atrial included), hydroelectrolytic disorders, hypoxia, postoperative pain, and genetic factors<sup>1-7</sup>.

Several strategies for reducing AF in the postoperative period after myocardial revascularization surgery

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CORRESPONDING AUTHOR: Marcel Pereira

Dr. Eneas Carvalho de Aguiar, 44, São Paulo, SP, Brasil, 05403-000

E-mail: marcelppereira@hotmail.com

(POAF) have already been tested. The prophylactic use of beta-blockers and amiodarone to prevent POAF is recommended by specific societies<sup>2,8</sup>.

In previous records, the incidence of POAF is more common in elderly patients, with lower ventricular function on echocardiography, and in patients who presented a greater number of comorbidities such as hypertension, diabetes, chronic obstructive pulmonary disease (COPD), kidney failure, among others<sup>4,5,9-11</sup>. Gillinov et al.<sup>3</sup> showed that there was no difference in the length of hospital stay in relation to strategies for pace control versus heart rate control for controlling AF. This same study found that the maintenance of the sinus rhythm from hospital discharge and for 60 days of follow-up was approximately 85%, regardless of the adopted strategy. In addition, the prescription of warfarin was mandatory if the arrhythmia lasted for more than 48 hours; however, the prescription only occurred in approximately 43% of the cases, and with an average duration of use for 44 days.

This arrhythmia increases hospital costs and the length of hospital stay<sup>4,9,12</sup>. In records from 49,264 patients<sup>6</sup>, the presence of POAF increased the rate of ICU stay in 48 hours (relative risk = 47.55), the length of hospital stay in 3 days (relative risk = 2.93), and the total hospital costs in 9,000 dollars (relative risk = 7.62). In addition, several records show that the patients who presented POAF had higher rates of outcomes in the short- and long-term. Steinberg et al.<sup>4</sup> demonstrated an increase in mortality and in the rate of cerebral vascular accident (CVA) during hospitalization and in 30 days of follow-up. In a meta-analysis with over 109,000 patients, Phan et al. demonstrated that POAF increased mortality in 30 days (relative risk = 1.95), as well as in-hospital complications and the CVA rate (relative risk = 2.06)<sup>6</sup>. Regarding the long-term outcomes, many publications have demonstrated an increase in mortality and thromboembolic events associated with this type of arrhythmia<sup>5,6,9-11</sup>. El-Chami et al.<sup>9</sup> assessed over 16,000 MRS, and POAF occurred in 18.5% of the cases. In the 6-year follow-up (with a difference in the first year), an increased risk of mortality was found. During that same follow-up, 20.5% of the patients in the POAF group were anticoagulated with warfarin and, in comparison to those who did not receive anticoagulants, in a multivariate analysis, the protective effect of this medication was demonstrated, with a reduction of 22% in mortality. The EXCEL<sup>10</sup> study compared percutaneous intervention strategies and elective MRS in patients with obstructive lesions

in the left coronary artery trunk. A subanalysis of this study, published in 2018, found a difference in the incidence of AF in the patients, with a prevalence of 18% in surgical patients and 0.1% in patients who underwent percutaneous interventions. POAF has been defined as AF lasting for over 30 seconds measured by electrocardiographic documentation or monitor, or the need for intervention in the arrhythmia. Upon hospital discharge, 85% of the patients had the arrhythmia resolved. In the 3-year follow-up, POAF was associated with increased rates of death, CVA, and in the composite outcome of death, acute myocardial infarction, and CVA. The prescription of anticoagulants occurred in 10.1% of the cases with POAF, all of which received warfarin<sup>10</sup>.

Thus, it is observed that POAF is, in most cases, a transient condition that may resolve itself spontaneously or after the prescription of medications to control the heart rate or rhythm. However, despite this, it is associated with clinical events such as death or CVA, increased time of hospitalization, and hospitalization costs. In addition, there is a recommendation for anticoagulation in these patients<sup>12,8</sup>. It is common practice to initiate anticoagulation in patients at a higher risk, which is defined as a CHA2DS2-VASC greater than or equal to 2 in patients who presented this arrhythmia for over 48 hours<sup>13</sup>. However, many records included POAF with duration shorter than 48 hours, demonstrating that the presence of this condition is already considered a risk for the patient during hospitalization, both in the short- and long-term. When it comes to anticoagulation strategies, there is data indicating that the prescription of such medication improves outcomes and is indicated in this scenario. However, recent studies have shown a low rate of prescription of this class of drug, and, when prescribed, the conventional prescription was of warfarin; thus, direct-acting oral anticoagulants (DAOs) were not tested in this scenario in large populations. Despite having already been validated in the context of *lato sensu* FA, DAOs require more evidence for being prescribed in this population. A pilot study<sup>14</sup> with edoxaban showed that the prescription of this medication in patients with POAF did not lead to higher rates of bleeding when compared with a placebo in a 2-months follow-up. In another retrospective study with 598 patients, 27.9% developed POAF. The prescription of anticoagulants occurred in 72 patients, 34.7% of which received DAOs. There was no difference in bleeding outcomes at the 30-day follow-up<sup>15</sup>.



Therefore, we believe that, although common and known to be associated with a worse prognosis, POAF is still a undertreated nosological entity. The prescription of anticoagulants for *lato sensu* AF is already well-established and, despite being recommended in a POAF scenario, its use in a real-world context remains with a certain inertia. American guidelines recommend prescribing anticoagulants for 2 to 3 months and, if the patient does not present recurrence of arrhythmia, the medication can be discontinued<sup>1</sup>. The European Society of Cardiology, in its guidelines for AF, recommends the anticoagulation without determining its duration<sup>2</sup>. The Society of Cardiothoracic Surgery recommends the anticoagulation for at least 4 weeks, in patients who

were discharged in AF<sup>8</sup>. We believe that the fact that warfarin is the default drug used, although difficult to manage, hinders the prescription of this class of drugs. We believe that anticoagulation should be prescribed during hospitalization and its maintenance reassessed after 30 to 90 days, actively searching for a recurrence of the arrhythmia based on clinical and Holter data, according to guideline recommendations. After that time, it is reasonable to consider the suspension of medication if the patient has a low risk of arrhythmia recurrence. In addition, we believe that the lack of evidence and/or access to DAOCs in this scenario also hinders the prescription of this class of drugs, thus more studies on these drugs are necessary.

**PALAVRAS CHAVE:** Procedimentos Cirúrgicos Cardíacos. Revascularização Miocárdica. Ponte de Artéria Coronária. Fibrilação atrial. Anticoagulantes.

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# COVID-19 and stroke: a thromboembolic hypothesis

 Renata R. T. Castro<sup>1</sup>  
 João Giffoni da Silveira Neto<sup>2</sup>  
 Roberta R. T. Castro<sup>2</sup>  
 Jacqueline Stephanie Fernandes do Nascimento<sup>3</sup>  
 Janie Kelly Fernandes do Nascimento<sup>3</sup>  
 Nicolle dos Santos Moraes Nunes<sup>3</sup>  
 Marco Orsini<sup>4,5</sup>

1. Professora titular na Universidade Iguaçu – UNIG, RJ, Brasil.

2. Faculdade de Medicina, Universidade Iguaçu – UNIG, RJ, Brasil.

3. Acadêmica de medicina na Universidade Iguaçu – UNIG, RJ, Brasil.

4. Médico, PhD pela Universidade Federal do Rio de Janeiro, Professor titular na Universidade Iguaçu – UNIG, RJ, Brasil.

5. Professor titular na Universidade de Vassouras – UniV-RJ, Brasil.

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KEYWORDS: COVID-19; Stroke; Thrombus; Hypercoagulability.

COVID-19 should be regarded as a systemic disease, with potentially lethal complications related to hypercoagulability. In fact, COVID-19 may induce the activation of the coagulation cascade through a cytokine storm<sup>2</sup>, and the increase in D-dimer and fibrin degradation products is linked to worse prognosis and mortality<sup>1</sup>.

Disseminated intravascular coagulopathy, venous thromboembolism, ischemic stroke, and pulmonary embolism represent the main thrombosis manifestations of COVID-19 hematologic disturbances. But other manifestations of hypercoagulability should not be neglected.

Hypercoagulability may also manifest as intra-ventricular thrombus. For instance, left ventricular thrombus is the consequence of Virchow's

triad in the ventricle<sup>4</sup>: reduced wall motion, local myocardial injury, and hypercoagulability. As blood stasis does not happen in ventricles with preserved function, and the formation of left ventricular thrombus is rare in individuals with normal systolic function. The incidence of left ventricular thrombus after ST-segment elevation myocardial infarction is 6.3%, but it increases to 19,2% when the left ventricular ejection fraction is reduced<sup>5</sup>.

A 70-year-old woman presented acute respiratory distress syndrome due to COVID-19. She had hypertension but was not in use of medications. Chest computerized tomography showed ground-glass opacities in 75% of both lungs. Laboratory results showed anemia (hematocrit: 31.5%; hemoglobin: 10.2 g/dL), lymphopenia (4%; 360/mm<sup>3</sup>) and low platelet count (84,000/

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CORRESPONDING AUTHOR: Renata R. T. Castro

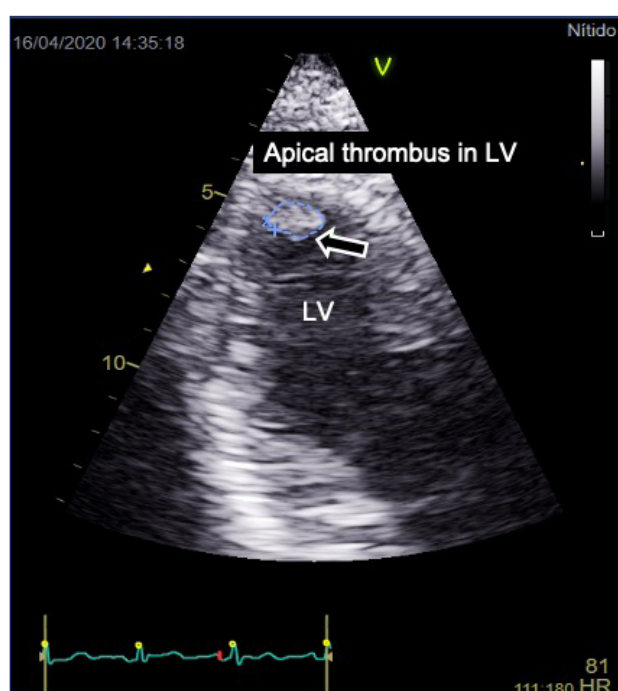
Rua Prudente de Morais, 1788, Ipanema, RJ, Brasil – 22420-042

E-mail: castrorrt@gmail.com

mm<sup>3</sup>). Both prothrombin time (13 s) and activated partial thromboplastin time (42,6 s) were within the normal range, and C-reactive protein was high (16.4 mg/dL).

Despite normal left ventricular ejection fraction (63%), normal left ventricular wall thickness, and normal left ventricular global longitudinal strain (-17,8%) there was a left apical ventricular thrombus (Figure 1). Anticoagulation with low molecular weight heparin was initiated, but the patient died suddenly on the 4<sup>th</sup> day of hospitalization.

**FIGURE 1.** ECHOCARDIOGRAM SHOWING APICAL THROMBUS (ARROW) IN THE LEFT VENTRICLE (LV)



The main risk of left ventricular thrombus is systemic embolism, especially cerebral. Patients with severe COVID-19 show a higher incidence of acute cerebrovascular diseases than the ones with mild disease<sup>3</sup>. Oxley et al<sup>6</sup> published a series of 5 cases of large vessel stroke in young patients with severe COVID-19. Coagulopathy and vascular endothelial dysfunction were appointed as causes for these strokes, but imaging of the head and neck did not reveal the source of the thrombus<sup>6</sup>. Given the present report, we would like to alert physicians in the frontline that cardioembolism should be considered as a potential mechanism for strokes in patients with COVID-19 infection.

Echocardiography and other imaging studies are not being frequently performed in patients with COVID-19 infection due to the risk of transmitting the infection to healthcare personal and other patients. Patient instability prevents their transportation from intensive care units. However, echocardiography has long been used as a point of care tool in intensive care. Noteworthy, patient position in intensive care may limit echocardiographic studies. Nevertheless, given the current setting, in which clinical decisions are based on the compassionate use of medications and assumptions, and considering that anticoagulation is not innocuous, an echocardiogram can help physicians to decide about anticoagulation regimens. The association between intracardiac thrombus and stroke in patients with COVID-19 infection remains to be proven in larger cohorts.

### Author's Contribution

All authors have contributed equally to this work

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# COVID-19: scars of the virus and stories caused by social isolation and mourning

 Claudia Terezinha Moraes Pinheiro Delgado<sup>1</sup>

1. Universidade do Vale do Itajaí, R. Uruguai, 458 – Centro, Itajaí, SC, Brasil.

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## SUMMARY

*We watch a variety of news on the Coronavirus daily. Some are reliable and others just instill more fear into the population. This article reflects on the possible consequences of this virus, which goes beyond the organic disease and the symptoms and signs mentioned daily in the media. Seeking a reflection on the effects caused indirectly by the virus. Since isolation precedes the disease, total isolation during the disease and isolation after the disease worsens, in some cases resulting in abrupt grief.*

**KEYWORDS:** Coronavirus, grief, social isolation, loss, respiratory infections.

In December 2019, the health authorities in the city of Wuhan, Hubei Province (China), reported individuals diagnosed with severe acute respiratory syndrome of unknown origin.<sup>1</sup>

On 30 January, the World Health Organization (WHO)<sup>2</sup> declared an international emergency, and approximately two months later, on 11 March,<sup>3</sup> declared it a pandemic.

In Brasil, up to 08 of April 2020, there had been 15,927 COVID-19 cases. In the last 24 hours, 2,210 new cases of the disease were confirmed, which represents an increase of 16% (2.210/13.717) in comparison to the total accumulated up to the previous day, according to the WHO and Johns Hopkins University.<sup>4</sup>

Viruses are considered etiological agents that both predispose individuals to secondary bacterial infections and are dominant agents of acute respiratory insufficiency. We have observed severe clinical manifestations associated with diseases of the lower respiratory tract in people with risk factors such as: heart

disease, lung disease among other chronic diseases such as hypertension, obesity, and diabetes.<sup>5,6</sup>

The Ministry of Health outlined as objectives of the SUS (Unified Health System) response to the Coronavirus disease, in its Epidemiological Bulletin, by the Center for Emergency Operations in Public Health, the following:

- Interrupting human to human transmission and mitigating events that broaden transmission;
- Timely identify suspected cases of flu and severe acute respiratory syndrome, based on clinical and laboratory diagnosis;
- Isolate symptomatic patients and provide care to patients;
- Promote studies to describe the natural history of the disease in Brasil, the pattern of transmission, severity, therapeutic options, the development of medicines and vaccines, in addition to contributing to the international effort to investigate gaps in the scientific knowledge about the disease;

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CORRESPONDING AUTHOR: Claudia Delgado

Rua Brusque, 920, Itajaí, SC, Brasil – 88302-202

Tel: +55 47 99602-8172

E-mail: claudiadelgadomestrado@gmail.com; claudiatmp@zipmail.com.br

- Establish responsive mechanisms to register, purchase nationally and/or internationally, distribute, evaluate and monitor the quality of products, equipment, and inputs related to the pandemic response so as to stimulate national production;
- Establish institutional guidelines for the management and sharing of scientific data and deployment of good practices of science and open data, ensuring transparency and monitoring by the society and control bodies of all decision-making processes, daily, the epidemiological situation, risk situation, and advances in response at each stage of the pandemic;
- Minimize the social and economic impact through multisector partnerships and in support of measures for expanded and selective social distancing adopted by the States, Federal District, and Municipalities;
- Monitor notified cases and deaths, occupations and installation of beds, supplies of personal protective equipment, laboratory tests (serological and molecular), mechanical respirators, labor force, logistics, and communication.<sup>4</sup>

What still has had little repercussions are the consequences of this virus in the people affected by it, both directly, when they contract the virus and are required to socially isolate, or when they lose a loved one without the possibility of farewells, a funeral, or any other symbolism, religious or not, that somehow helps to deal with the mourning process.

Managing to juggle and minimize the economic, social, and psychological damages to the population by adopting fiscal and social measures is essential.<sup>7,8</sup> What we seek as citizens is managers prepared to work in these areas, enabling health professionals and able to act in an interdisciplinary way.

Social isolation has already demonstrated to leave scars in previous times, as occurred in the mid 20<sup>th</sup> century, when the country, with the intention of controlling and ending “leprosy”, adopted the strategy of compulsory isolation. So, Hansen’s disease patients were removed from public life, believing that by isolating the sick a healthy society would be preserved. Many people who were only suspected of having the disease, based on complaints from neighbors, were taken from their homes, which were later burned, suffering all the embarrassment caused by hospitalization.<sup>9</sup>

The suffering resulting from the social isolation of those people suffering from or suspected of having Hansen’s disease, combined with the need for care, would mobilize them toward overcoming an experience of social segregation. Obviously, the different ways of coping depend on the perception of each individual and vary according to individual experiences. However, we can learn something from that past experience that is similar to what we are currently experiencing.

The virus, the social awareness, the people, the government, the latter not always, and fear itself make us isolate, either individually or in a “family”, in small family bubbles. In the effort to not contract the virus, or to not pass it along to our loved ones, and in the expectation of a possible cure or protection.

The first consideration we report here is that we know that the feeling of isolation and lack of social support is a risk factor for suicide recognized by the World Health Organization.<sup>10,11</sup> Suicide rates inversely vary with the degree of social integration of an individual. Studies have shown that unmarried individuals are 38% more likely to commit suicide. Recent migrants and people without religion also compose the variables identified as of risk for suicide.<sup>10</sup>

We must be vigilant and think about creating programs so that everyone can have social interaction, even if through technology, and by that we mean actual soft-hard technology, as in those available by computer, so that we can work on the soft technology, which would be providing refuge to others. Seek ways to integrate so we can overcome this isolation, whether it is temporary or not.

The current guidelines establish that after testing positive by RT-PCR or serological tests, individuals should be put on a 14-day leave from work. If it is not possible to get tested, but flu syndrome (symptoms) is present, guidelines establish the prescription of a 7-day leave from work, starting at the onset of symptoms, with a return to work after seven days, if the individual remains asymptomatic. Serological tests should be carried out on the eighth day after the onset of symptoms.<sup>4</sup>

Having explained the current guidelines, we present the second consideration, which regards the prejudice suffered by those who received a diagnosis or were given leave from work for presenting symptoms and find themselves isolated by everyone. This demonstrates that even if these individuals do not present symptoms or the possibility of transmission, they will

remain in isolation, either by fear and insecurity that they can still transmit the virus or by the fear of others of contracting it.

And to those who lost relatives, there is the impossibility of a farewell, whether in life or in death. What are the long-term consequences that a disease with such transmission capacity and abrupt development can leave to those who continue living?

Death, in the Western world, is a taboo, surrounded by mysteries and beliefs, and people are often unprepared to deal with the human finitude. When a death occurs tragically and suddenly, it tends to cause numerous changes in a person's life, causing, often-times, damage and changes, particularly in emotional and cognitive functions.<sup>12</sup>

There are reports, according to Parkes,<sup>13</sup> that people who lost a loved one, suddenly, wept more, felt numb and missed the loved one more than those who also lost a loved one but in other circumstances. It was also observed that the sudden loss of children, in car accidents, caused in their parents more depression, anger, and guilt and, consequently, more health problems, in addition to painful memories of the person who died, than when a child dies due to a disease of which the parents are aware. In these descriptions, it is possible to note that sudden, unexpected, and early death is considered a complicating predictor to the usual mourning process and may lead to psychological problems such as depression and anxiety.<sup>12,13</sup>

Those in grief are left with the option of resorting to a psychologist, who also now provide remote care, by telephone, in order to provide refuge and listening to the patient. Follow-up with a psychologist can contribute to improvements in coping with this process. Considering that people experiencing the loss

of a loved one are trying to find reasons, the details of the death (how it was, where it was), in their effort to find a rational understanding of how it happened, this is essential to alleviate their pain, anxiety, and confusion.<sup>12</sup>

## FINAL CONSIDERATIONS

Given the scenario set out in this paper regarding ongoing social isolation and the possible sudden death of an individual or their family and acquaintances, all of which bring consequences as stressful life events, caused by a virus that removes people from their core and context, what is left, beyond doubt, is loss and isolation that lead to suffering and of great psychological changes. It is necessary to have managers who are attentive to needs that are changing and who can creatively provide adjustments to deal with these new financial, emotional, social, and affective shortcomings. Society needs to learn how to move forward and provide refuge to all those who went through this isolation, i.e. Society as a whole, globally, since this is a pandemic virus.

Those in mourning require increasingly more the assistance of a psychologist, a health professional who will assist them by listening and providing refuge to the weakened human being who survived. More studies and reflections deserve to be shared so that we can together not feel so alone, sharing knowledge and possibly different contexts, which involve several countries. Since there are not many Brazilian studies that address the grief generated by a sudden loss in this proposed references, i.e., on the impossibility of a farewell, it is important to highlight that new research should be carried out, and we can provide interested professionals updated materials on this topic.

## RESUMO

*Observamos diariamente uma variedade de notícias sobre o Coronavírus. Algumas confiáveis e outras apenas para causar mais temor para a população. Este artigo realiza uma reflexão sobre possíveis consequências desse vírus, que vai além da doença orgânica e dos sintomas e sinais referidos diariamente nos meios de comunicação. Buscando uma reflexão sobre efeitos ocasionados indiretamente pelo vírus. Sendo o isolamento antecedente a doença, isolamento total durante a doença e isolamento posterior a agudização da doença, em alguns casos resultando em luto abrupto.*

**PALAVRAS CHAVE:** Coronavírus, luto, isolamento social, perdas, infecções respiratórias.

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# Home Care as a safe alternative during the COVID-19 crisis

 Heloisa Amaral Gaspar<sup>1</sup>  
 Cláudio Flauzino de Oliveira<sup>2</sup>  
 Fabiana Camolesi Jacober<sup>3</sup>  
 Eduardo Roberto de Deus<sup>4</sup>  
 Flavia Canuto<sup>5</sup>

1. Médica Intensivista Pediátrica, Doutora em Ciências pela Faculdade de Medicina da USP, Gerente Médica Home Doctor, São Paulo, Brasil.
2. Médico Intensivista Pediátrico, Doutor em Ciências pela Faculdade de Medicina da USP, Diretor Home Doctor, São Paulo, Brasil.
3. Enfermeira especialista em Controle de Infecção Domiciliar e Analista de Qualidade Home Doctor, São Paulo, Brasil.
4. Analista Sênior de Qualidade Home Doctor, São Paulo, Brasil.
5. Enfermeira especialista em Gerontologia, Gerente Comercial Home Doctor, São Paulo, Brasil.

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## SUMMARY

**INTRODUCTION:** *There are several reports worldwide about the high mortality related to COVID-19 among residents of nursing homes. The worldwide concern about the safety of patients and professionals in these institutions is relevant. In Brasil, a large part of post-acute care and chronic patients is performed at home through Home Care (HC).*

**OBJECTIVE:** *This study aims to evaluate the incidence of COVID-19 in Home Care patients and the clinical outcomes of these patients; it also aims to assess the impact of the epidemic on the number of patients, new admissions, and hospitalizations.*

**METHODS:** *A descriptive study of the COVID-19 cases that affected the population in care by Home Doctor (a private company of Home Care), between the months of March 2020 and May 2020 and analysis of the total number of patients, the hospitalization and death rate in the period compared to the pre-epidemic period.*

**RESULTS:** *There were 31 confirmed cases of COVID-19, 21 of which were male, mean age 73 years. All patients had multiple comorbidities, the most prevalent were: Systemic Arterial Hypertension (54%) and Stroke (35%). The incidence of COVID-19 was 1% in the studied population. There were 10 hospitalizations with 5 hospital deaths and one case of home death (lethality 19%). Safe care was maintained, with a low death rate (0.6%) and hospitalization (6.1%).*

**CONCLUSION:** *Home Care is able to maintain safe care during the pandemic due to COVID-19, with a low incidence of COVID-19, low hospitalization rate, and low mortality when compared to nursing homes institutions.*

## INTRODUCTION

The World Health Organization (WHO) received, in December 2019, the first reports of cases of pneumonia of unknown etiology in the city of Wuhan (Hubei province, China), subsequently attributed to infections by the new betacoronavirus called SARS-CoV-2, Severe

Acute Respiratory Syndrome Coronavirus 2. On 30 January, the WHO declared it a global public health emergency, putting the whole world on alert. On 11 February, the new disease was named COVID-19. Patients infected by SARS-CoV-2 were identified in

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CORRESPONDING AUTHOR: Heloisa Gaspar

Rua Doutor Jose Aureo Bustamante, 301, apto 232-C, São Paulo, SP, Brasil – 04710-090

Tel: +55 11 99313-3902

E-mail: heloisa.gaspar@homedoctor.com.br



other countries, mainly in Europe, in the United States (USA), in Canada, and in Brasil. On 11 March 2020, the WHO characterized COVID-19 as a pandemic.

In Brasil, the first confirmed case of COVID-19 occurred on 26 February. On 20 March, the Ministry of Health acknowledged, through Decree No 454, the status of community transmission of COVID-19 throughout the national territory. Since then, the number of cases has grown exponentially, and currently, despite the known underreporting, the country holds the second position worldwide among countries with the greatest number of cases and deaths due to COVID-19.<sup>1</sup>

There are various reports worldwide on the high mortality related to COVID-19 among the residents of Nursing Homes, which correspond to 25% of all deaths related to COVID-19 in the United States, and even larger percentages in some North American states and European countries (such as France and Ireland)<sup>2</sup>. The worldwide concern regarding the safety of patients and professionals in these institutions is entirely appropriate.

In Brasil, unlike the scenario of other countries such as the USA, a large part of post-acute care, rehabilitation and care of chronic patients are performed at home, through the home care (HC), instead of in nursing homes, both in the public sector and in complementary health. This modality includes the administration of medicines, enteral nutrition, wound dressing, rehabilitation therapies, oxygen therapy, and even more complex therapies such as parenteral nutrition and invasive and non-invasive mechanical ventilation.

## OBJECTIVE

The present study aims to evaluate the incidence of COVID-19 in patients undergoing home care and the clinical outcomes of these patients; in addition, we aim to assess the impact of the epidemic on the numbers of total patients, new admissions, and hospitalizations during the initial period of the pandemic.

## METHODS

This is a descriptive study that included the collection, tabulation and analysis of data on COVID-19 cases that affected a population undergoing care by Home Doctor (a private home care company), between March 2020 and May 2020, i.e., the 1<sup>st</sup> quarter of

the epidemic (1QTREPI), reporting the incidence of COVID-19 cases in this population and analyzing the clinical data and outcomes of these patients.

We analyzed the total number of patients undergoing care, the number of new cases (related and unrelated to COVID-19), as well as the rate of hospitalization and death in the 1QTREPI in comparison to January/2020, using as a reference the period pre-epidemic (REFPRE).

The data were retrieved from the electronic medical records system of the company and presented as absolute numbers (N), frequency (%), and mean when appropriate.

## RESULTS

In the 1QTREPI, 2931 patients were treated in all home care programs. The company recorded over this period 31 confirmed COVID-19 cases, 10 of them in females and 21 in males, with a mean age of 73 years (21-95 years). All patients presented multiple comorbidities, and the most prevalent were: Systemic Arterial Hypertension in 17 cases (54%) and sequelae from strokes in 11 cases (35%). The incidence of COVID-19 was 1% in the population studied. There were 10 hospitalizations with 5 in-hospital deaths and one case of death at home, a patient undergoing exclusive palliative care, which represents a mortality rate of 19% (Table 1).

The geographical distribution of confirmed COVID-19 cases was disclosed on a daily basis for the entire care team through a *case map*, and data relating to 31/05 are presented in Figure 1.

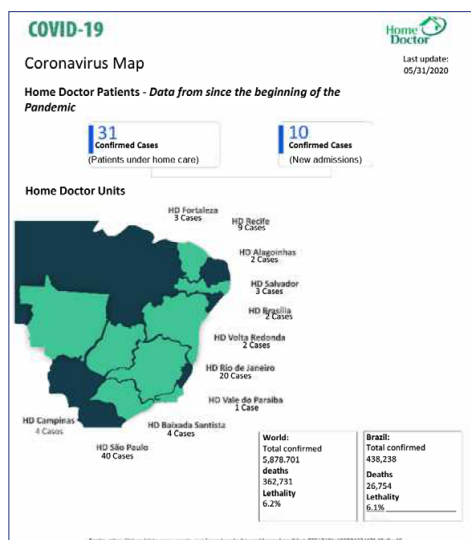
Virtual consultations have been made available with doctors, nurses, nutritionists, psychologists and social workers since April/2020 aiming to reduce the commuting of professionals to patient homes and, consequently, reduce patient exposure (54% of medical visits and 44% of nurse visits were replaced by telehealth consultations). Physiotherapy and speech therapy sessions were maintained in-person due to the profile of the need of the population. A satisfaction survey was applied in patients treated through this modality using the Net Promoter Score (NPS), with a result of 54% (zone of quality).

Patients undergoing home care received safe and quality care, with their clinical stability maintained, which can be evidenced by a low mortality rate and a low and declining rate of hospitalization in the 1QTREPI (Table 2).

**TABLE 1.** CLINICAL DATA OF PATIENTS WITH A DIAGNOSIS OF COVID-19 UNDERGOING HOME CARE

| Initials | Age | Gender | Program | Morbidity background   | Clinical outcomes |
|----------|-----|--------|---------|--|-------------------|
| JSP      | 85  | MEN    | HC      | CHF, DM, SAH, Arrhythmia   | Hospitalization   |
| WGL      | 45  | MEN    | HS      | AIDS (HIV), Nasal neoplasia with metastasis in cervical lymph nodes    | Recovered         |
| JSPF     | 78  | MEN    | HC      | Alzheimer's, SAH   | Recovered         |
| OGM      | 74  | MEN    | HS      | COPD   | Recovered         |
| WAVM     | 64  | MEN    | HS      | CVA, Hydrocephalus, SAH  | Recovered         |
| FACM     | 71  | MEN    | HC      | Parkinson's, Coronariopathy  | Recovered         |
| MJPB     | 91  | WOMEN  | HC      | SAH, CHF, COPD, CVA  | Recovered         |
| MLPG     | 88  | WOMEN  | HS      | Parkinson's, Hypothyroidism  | Recovered         |
| RMR      | 84  | MEN    | HS      | Dementia, Chronic atrial fibrillation                                  | Recovered         |
| TSP      | 30  | WOMEN  | HC      | Genetic Sd (HSAN), Paced bradycardia                                   | Recovered         |
| EG       | 62  | MEN    | HC      | Obesity, Asthma, SAH, Hip prosthesis, PTE                              | Recovered         |
| LGP      | 95  | WOMEN  | HC      | Alzheimer's, SAH, CVA  | Recovered         |
| SF       | 90  | MEN    | HS      | Parkinson's, Hypothyroidism  | Recovered         |
| FJGM     | 57  | MEN    | HS      | CVA, SAH, Obstructive sleep apnea                                      | Recovered         |
| AJAL     | 71  | MEN    | HS      | CVA, Sigmoid volvulus, SAH   | Death at home     |
| NRG      | 93  | WOMEN  | HC      | Dementia+ DM + SAH + AMI + CHF + CKD                                   | In-hospital death |
| JGA      | 74  | MEN    | HC      | COPD, DM   | In-hospital death |
| HAT      | 90  | MEN    | HS      | CVA, Dementia, DM type II, SAH   | In-hospital death |
| NB       | 77  | WOMEN  | HC      | Aortic aneurysm, CVA, Hypothyroidism.                                  | In-hospital death |
| MBL      | 91  | WOMEN  | HS      | CVA, Thrombophilia, Lower limb arterial thrombosis, PTE, COPD, SAH, DM | In-hospital death |
| HT       | 82  | MEN    | HC      | SAH, Dementia, Parkinson disease, CVA                                  | Recovered         |
| ASVC     | 85  | WOMEN  | HS      | SAH, CHF, Parkinson disease  | Recovered         |
| MJPB     | 86  | MEN    | HC      | SAH, Coronariopathy, CKD   | Recovered         |
| IRM      | 83  | MEN    | HS      | Alzheimer's disease  | In treatment      |
| RVPS     | 40  | MEN    | HS      | TBI + Chemical dependency (cocaine)                                    | Recovered         |
| LPL      | 85  | WOMEN  | HC      | Obesity, SAH, DM   | Hospitalization   |
| REM      | 42  | MEN    | HS      | Epilepsy, CNPE   | Hospitalization   |
| LASR     | 21  | MEN    | HS      | CNPE + DM + SAH + Asthma   | In treatment      |
| RMF      | 75  | MEN    | HC      | Obesity, SAH, CVA, Polyneuropathy                                      | In treatment      |
| LBLS     | 84  | WOMEN  | HC      | DM   | In treatment      |
| MGS      | 79  | MEN    | HS      | SAH, Coronariopathy, CVA   | Hospitalization   |

HC: Home care, HS: Home Stay; CHF: Congestive Heart Failure; DM: Diabetes Mellitus; SAH: Systemic arterial hypertension; AIDS: Acquired immunodeficiency syndrome; COPD: Chronic obstructive pulmonary disease; CVA: Cerebrovascular accident; PTE: Pulmonary thromboembolism; CKD: Chronic kidney disease; TBI: Traumatic brain injury; and CNPE: Chronic non-progressive encephalopathy.

**FIGURE 1.** CASE MAP AND GEOGRAPHICAL DISTRIBUTION**TABLE 2.** NUMBER OF PATIENTS, HOSPITALIZATION RATE, AND DEATH RATE OF PATIENTS UNDERGOING HOME CARE IN THE PREEPI AND 1QTREPI PERIODS

| Home Doctor Group    | Jan/20 | Feb/20 | Mar/20 | Apr/20 | May/20 |
|----------------------|--------|--------|--------|--------|--------|
| Number of patients   | 1974   | 2033   | 2115   | 2213   | 2279   |
| Hospitalization rate | 11.00% | 9.30%  | 8.80%  | 6.90%  | 6.10%  |
| Death rate           | 0.60%  | 0.60%  | 0.60%  | 0.32%  | 0.60%  |

From January to May, we observed an increase of 305 patients/month in home care, which represented an increase of 15% in cases treated (Table 2). This increase in patients can be attributed to:

I - Hospital discharge of COVID-19 patients, either in an active stable phase of the disease or in the rehabilitation phase

During the 1QTREPI, a total of 35 patients were admitted into home care, from a hospital, with a diagnosis of COVID-19, 11 (32%) of them still in the active acute phase of the disease, and 24 (68%) for rehabilitation therapy and treatment of complications.

II - Hospital discharge of with diagnoses unrelated to COVID-19

During the 1QTREPI, 1137 patients were submitted to home care from hospitals for 90 days. The main reasons for home care were the need for end of treatment with intravenous, intramuscular or subcutaneous medication, oxygen therapy, dressings, nutritional support, and rehabilitation therapies.

III- To avoid hospitalization of suspected or confirmed non-critical cases of COVID-19, through home monitoring of oximetry, oxygen supplementation, medical follow-up at home, daily medical telephone monitoring, and emergency medical center available 24/7.

In the 1QTREPI, a total of 76 patients were monitored with confirmed or suspected COVID-19, with 10 hospitalizations (13%).

## DISCUSSION

The home care industry grew exponentially in recent decades. Currently, it is estimated that approximately 1 million patients start home treatment yearly in Brasil, including the public and private sectors<sup>3,4</sup>. Thus, this segment is responsible for reducing the demand for hospital beds and the overload of the hospital sector, something that becomes even more important in the context of the COVID-19 epidemic.

Advanced age and the presence of comorbidities are associated with increased mortality in the pandemic caused by the novel coronavirus. The high prevalence of this combination, associated with physical environments that provide inadequate barriers for infection control, puts nursing home patients at great risk, and studies have shown that once a first case occurs in these institutions, the ability of the infection to spread to other patients is quite high<sup>5,6,7</sup>. The world literature reveals alarming figures regarding these institutions, with 2/3 of patients affected in a period of 3 weeks and mortality rates reaching values as high as 72%<sup>8</sup>.

Home care, which is classically recognized by the social benefits it provides, such as patient participation in family life and society and consequent improvement in the quality of life, presents a unique advantage in terms of patient safety and infection control. The patient is naturally maintained in home isolation,

assisted by a team of professionals trained to meet the special need of their care. This, in association with the correct use of individual protection equipment (IPE) and incorporation of innovations in the sector (such as the use of telehealth) are key points for safe healthcare in the context of the epidemic<sup>9,10,11</sup>.

The study population consisted primarily of elderly patients with comorbidities, and in this known risk group, the incidence of COVID-19 was of only 1%. The subgroup of patients affected by COVID-19 was 80% comprised of elderly individuals, all of them with comorbidities, and the mortality of 19% observed is quite lower than that reported worldwide in nursing homes.

The epidemic drove the rapid incorporation of telehealth in the context of home care, with patient satisfaction within the quality level set by the NPS. Learning about this technology, its risks and benefits, will be essential so that professional councils and societies can define the role of this tool in the future.

Home care, like all health sectors in Brasil and worldwide, faced difficulties with the emergence of the epidemic regarding the supply chain (with scarcity and overpricing of inputs), interruption or reduction of public transport that hindered the commuting of professionals, increased rate of absenteeism, need for collaborator leaves, among others. However, it showed great plasticity and the ability to adapt, quickly and effectively re-structuring itself and being able to keep patients safe in their homes while actively contributing to keeping hospital beds available.

## CONCLUSION

Home care has proven to be able to treat patients of different complexities in their home environment safely during the COVID-19 pandemic, with a low incidence of COVID-19, low hospitalization rates, and low mortality when compared to nursing homes.

Home care, in addition to maintaining patients safely in their households, is capable of increasing its capacity rapidly, admitting patients from hospital environments and contributing to improve the availability of hospital beds.

The current pandemic has led to great considerations on how to provide the best health care and create safe treatment options beyond the hospital environment. The migration of post-acute and chronic care to the home environment with the use of technology seems to be an important piece to solve this complex puzzle.

## RESUMO

**INTRODUÇÃO:** Diversos são os relatos mundiais a respeito da elevada mortalidade relacionada a COVID-19 entre os residentes de instituições de longa permanência. A preocupação demonstrada mundialmente sobre a segurança dos pacientes e profissionais nestas instituições é relevante. No Brasil grande parte dos cuidados pós-agudo e de pacientes crônicos é realizado no domicílio através da Atenção Domiciliar (AD).

**OBJETIVO:** O estudo visa avaliar a incidência de COVID-19 em pacientes em Atenção Domiciliar e o desfecho clínico destes pacientes; objetiva avaliar o impacto da epidemia sobre número de pacientes totais, novas admissões e hospitalizações.

**MÉTODOS:** Estudo descritivo dos casos de COVID-19 que acometeram população em atendimento pela Home Doctor (empresa privada de Atenção Domiciliar), entre os meses de março de 2020 a maio de 2020 e análise do número total de pacientes, da taxa de hospitalização e óbito no período comparado o período pré-epidemia.

**RESULTADOS:** Ocorreram 31 casos confirmados de COVID-19, sendo 21 sexo masculino, idade média 73 anos. Todos os pacientes apresentavam múltiplas comorbidades, sendo as mais prevalentes: Hipertensão Arterial Sistêmica (54%) e Acidente Vascular Cerebral (35%). A incidência de COVID-19 foi de 1% na população estudada. Ocorreram 10 hospitalizações com 5 óbitos hospitalares e um caso de óbito domiciliar (letalidade 19%). A segurança do atendimento foi mantida, com baixa taxa de óbito (0,6%) e hospitalização (6,1%).

**CONCLUSÃO:** A Atenção Domiciliar é capaz de manter atendimento seguro durante a pandemia por COVID-19, com baixa incidência de COVID-19, baixa taxa de hospitalização e baixa mortalidade quando comparada a instituições de longa permanência.

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# Inflammatory cytokines and lipid profile in children and adolescents with nephrotic syndrome receiving *L. Plantarum*: a randomized, controlled feasibility trial

 Patrícia Marques Fortes<sup>1</sup>  
 Ricardo Vieira Teles Filho<sup>2</sup>  
 Lucas Henrique Souza de Azevêdo<sup>2</sup>  
 Victória Coelho Jácome Queiroz<sup>2</sup>  
 Paulo Sérgio Sucasas da Costa<sup>1</sup>

<sup>1</sup>. Departamento de Pediatria, Hospital das Clínicas, Faculdade de Medicina, Universidade Federal de Goiás (UFG), Goiânia, Goiás, GO, Brasil.  
<sup>2</sup>. Faculdade de Medicina, Universidade Federal de Goiás (UFG), Goiânia, Goiás, GO, Brasil.

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## SUMMARY

*This study aimed to evaluate the efficacy of the action of the Lactobacillus Plantarum probiotic as a immunomodulatory and hypolipidemic agent in dyslipidemic nephrotic children and adolescents.*

**METHODS:** *This is a randomized, double-blind, placebo-controlled clinical trial in pediatric, compensated or partially compensated nephrotic syndrome and dyslipidemic subjects undergoing regular outpatient follow-up. Serum lipid and TNF- $\alpha$  (proinflammatory) and IL-10 (anti-inflammatory) cytokine variations were evaluated. Cytokines were analyzed by enzyme-linked immunosorbent assay (ELISA).*

**RESULTS:** *In the probiotic group there was a tendency to reduce TNF- $\alpha$  levels and increase IL-10 levels when compared to controls. Regarding the lipid profile, there was a decrease in serum triglyceride (6.0 mg / dL) and total cholesterol (41.5 mg / dL) levels in the probiotic group when compared to baseline levels, while in the control group there was an increase in serum triglyceride (49.5 mg / dL) and total cholesterol (8.0 mg / dL) levels, respectively.*

**CONCLUSION:** *Preliminary results suggest that *L. Plantarum* showed an immunomodulatory and hypolipidemic effect in nephrotic and dyslipidemic pediatric subjects.*

**KEYWORDS:** *Child. Inflammation. Probiotics. Hypercholesterolemia. Nephrotic Syndrome.*

## INTRODUCTION

Nephrotic syndrome (NS) is a clinical entity characterized by changes in glomerular permeability that result in massive proteinuria, hyperlipidemia, hypoalbuminemia, and generalized edema<sup>1</sup>. The incidence of NS in childhood is estimated to be 4.7 (1,15 - 16.9) per 100,000 children worldwide. In children, the most

common histopathological alteration of NS is called “minimal change disease” (MCD) and is characterized by a good response to corticosteroids in about 80-90% of the cases<sup>2</sup>.

In the past decades, biomolecular research determined functional alterations of the immune system

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CORRESPONDING AUTHOR: Ricardo Vieira Teles Filho

Faculdade de Medicina – Universidade Federal de Goiás (UFG) Av S/N, Setor Universitário, Goiânia, Goiás, Brasil – CEP: 74-605-050

E-mail: ricardovteles@gmail.com



were important components of NS pathophysiology. The acute phase of MCD is characterized by an imbalance in T-cell subpopulations, with a predominance of suppressor T cells (CD8+) and T helper cells (Th-1 and Th-2). These cells produce cytokines such as interleukins (IL-1, IL-2, IL-4, IL-12, IL-13, IL-18), tumor necrosis factor-alpha (TNF- $\alpha$ ), and tumor growth factor-beta (TGF- $\beta$ )<sup>3</sup>, which is a trigger for starting and aggravating inflammation<sup>4</sup>. In addition, the participation of monoclonal B cells, immune complexes deposition on glomerular basement membrane (GBM), antigen response, and the presence of antibody receptors located on the surface of glomerular cells are also some of the mechanisms by which the immune system alters the electronegativity and permeability of the GBM, leading to proteinuria<sup>2,5,6</sup>. Hence, the available treatments for SN act on blocking this activation of the immune system.

On the other hand, the action of the immune system in altering the glomerular filtration barrier leads to hypoalbuminemia. This activates hepatic lipoprotein synthesis, which, in the long term, results in dyslipidemia. Thus, patients with SN have increased serum cholesterol levels, triglycerides, intermediate-molecular-weight lipoproteins (IDL), low-molecular-weight lipoproteins (LDL), and apolipoprotein B containing very-low-molecular-weight lipoproteins (VLDL). The persistence of hyperlipidemia in nephrotic individuals is known to play an important role in maintaining inflammation, increasing cardiovascular risk and liponephrotoxicity, accelerating the progression of renal injury. Therefore, for individuals with NS, the binomial dyslipidemia/proteinuria is particularly severe, because the overlapping of factors capable of activating the immune cascade favors the emergence of mechanisms that trigger vascular and renal injury<sup>6</sup>.

Just like inflammation and hyperlipidemia should be treated, there is a growing amount of evidence pointing to a direct and bi-directional interaction between the microbiota and the pathophysiology of renal lesions. On the one hand, progressive uremia may promote changes in metabolism and microbiota composition; on the other hand, many toxins produced by such bacteria (altered microbiota) increase renal injury<sup>7,8</sup>. For this reason, the use of probiotics has been drawing attention as concomitant therapy.

Studies demonstrating the impact of the intestinal microbiota on the health of the host have gained scientific interest regarding the use of probiotics as a therapeutic option in several different health problems<sup>9-11</sup>.

Regarding the possibility of its use in NS, two of the different mechanisms of action of probiotics stood out: 1) the ability to exert immunomodulatory effects when interacting with epithelial, dendritic, monocyte, and macrophage cells<sup>12</sup>, considerably influencing various aspects of the immune system by improving granulocyte phagocytosis, stimulating cytokine secretion in lymphocytes, and modifying T-cell responses (improving Th1 responses and attenuating Th2 responses)<sup>12,14</sup>; and 2) its hypolipidemic ability through enzymatic production (bile salt hydrolase), incorporation of cholesterol into the bacterial cell membrane, inhibition of cholesterol transporter in the enterocytes, and inhibition of hepatic cholesterol synthesis<sup>15</sup>.

Therefore, given the complex pathophysiology related to NS and due to these characteristics presented by probiotics, the objective of this paper is to evaluate the efficacy of the *Lactobacillus Plantarum* probiotic as a immunomodulatory and hypolipidemic agent in dyslipidemic children and adolescents with nephrotic syndrome.

## METHODS

This study consists of a randomized, double-blind, placebo-controlled clinical trial in children and adolescents with nephrotic syndrome (NS), under regular outpatient follow-up for more than 1 year at the referral university hospital in the pediatric nephrology service of the Center-West region of Brasil. The research project was approved by the Research Ethics Committee (n° 1.733.937) and registered in the Brazilian Registry of Clinical Trials (ReBEC – RBR-36byz5).

### Selection and sample calculation

The population consisted of children and adolescents with SN, according to the International Study of Kidney Disease in Children (ISKDC)<sup>16</sup> criteria, who were frequent, compensated or partially compensated, and who had dyslipidemia despite the frequent treatment of nephrotic syndrome, and attended the service from June/2016 to July/2017.

For the sample calculation, we used the statistical significance of 5% ( $p < 0.05$ ) and the power of the two-tailed test at 80%. As a reference for the calculation, the data presented in the study by Fuentes et al.<sup>17</sup> was used as a model. For the sample calculation, the results of total cholesterol and LDL-c after the use of *L. Plantarum* or placebo were described as mean and standard error and converted to standard deviation.

Thus, the OpenEpi<sup>18</sup> version 3.01 program was used to perform the calculation, including a 20% loss potential ( $10 + 20\% = 12$  patients in total), resulting in a total sample of 12 individuals - 6 in the *L. Plantarum* group (intervention) and 6 in the control group.

### Inclusion and exclusion criteria

Individuals aged 2 to 17 years old, regardless of gender, with MCD or focal and segmental glomerulosclerosis (FSGS), who presented in the last 3 months: 24h proteinuria  $\leq 50$  mg/kg/day or  $<40$  mg/m<sup>2</sup> body surface area (BSA)/hour, serum albumin  $< 3.0$  g/dl, total cholesterol  $\geq 170$  mg/dl and triglycerides  $\geq 130$  mg/dl, preserved renal function and on or off corticosteroids (dose  $\leq 1$ mg/kg/day) and/or cyclosporine (dose  $\leq 5$ mg/kg/day). Individuals who had: infectious processes or clinical and/or laboratory decompensation of NS less than 3 months from the beginning of the study, chronic kidney disease stage III or higher, any chronic systemic disease (hypothyroidism, diabetes mellitus, heart disease, lupus) were not admitted, neither were those who were on statins or other hypolipidemic drugs (phytosterols, prebiotics, symbiotics).

### Randomization of the groups

The selected individuals were numbered from 1 to 12 and divided into three groups containing 4 numbers, respectively. Using the software available on [www.randomization](http://www.randomization), the numbers ordered the patients to be enrolled in the study.

### Intervention and control characteristics

The intervention group used the probiotic *Lactobacillus Plantarum*, strain Lp-G18, (Lemma Supply Solutions), purchased as a lyophilized powder and compounded into gelatin capsules, containing  $2.5 \times 10^9$  CFU/capsule. For the control, 200 mg starch capsules were compounded as the placebo, produced with the same physical characteristics presented in the probiotic capsules, both stored in identical bottles containing 90 capsules.

### Study design

The study was conducted in parallel, divided into 2 groups: a probiotic group, i.e., patients who received *L. Plantarum* orally in capsules containing 2.5 billion CFU, 2x/day for a period of 12 weeks<sup>17</sup>, and a control group, i.e., individuals who received placebo oral capsules containing 200 mg of starch 2x/day, for another period of 12 weeks.

Subjects underwent four clinical evaluations throughout the study: T0 - selection evaluation, application of informed consent form, laboratory tests and guidelines on the removal of fermented foods from the diet; T2 - randomization and distribution of medication; T3 - laboratory evaluation, checking of medication in use and counting of dispensed medication, distribution of new bottle; T4 or endpoint - laboratory evaluation, dispensed medication count and last collection of laboratory tests.

Evaluations were performed every 45 days and consisted of checking weight, height, blood pressure, waist circumference, and certification of adverse events. They were then referred to the laboratory for evaluation of urine test, 24-hour proteinuria, urea, creatinine, serum albumin, total cholesterol, LDL cholesterol, HDL cholesterol, triglycerides, TNF- $\alpha$ , and interleukin 10 (IL-10). The dosages of TNF- $\alpha$  and IL-10 were measured by enzyme-linked immunosorbent assay (ELISA).

### Statistical evaluation

A database was created using Microsoft Excel version 2010 and Statistical Package for Social Sciences (SPSS<sup>®</sup>) 20.0 for Windows. Descriptive and bivariate analyses were performed considering the significance level of 5% ( $p < 0.05$ ). The results were submitted to the following statistical tests, taking into consideration the nature of the studied variables: a) measures of central tendency and variability - mean, standard deviation ( $\pm$  SD) and mean confidence interval (95% CI) being evaluated by descriptive statistics; b) Mann-Whitney U test - was used to compare numerical variables of apparently non-normal distribution.

## RESULTS

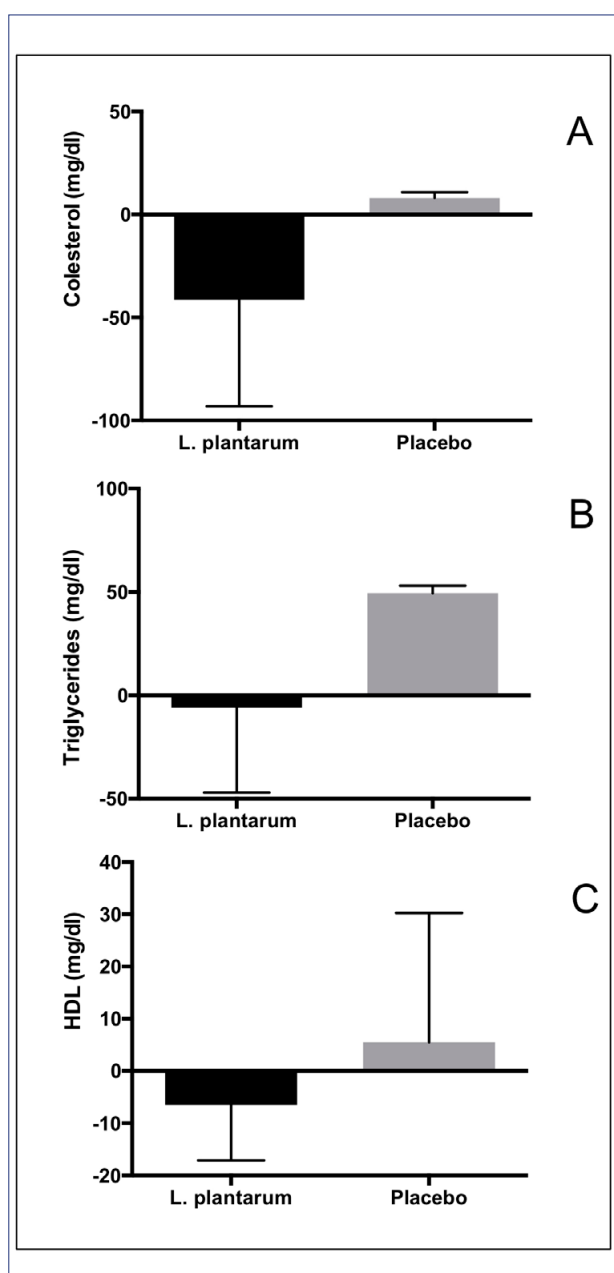
A total of 10 patients were selected, of which 4 met the inclusion criteria. The mean age ranged from  $9 \pm 2.82$  years for the probiotic group and  $8.5 \pm 6.36$  years for the control group. Each group consisted of 1 boy and 1 girl. Table 1 summarizes the pre-intervention parameters of the probiotic and control groups for serum levels of total cholesterol, LDL, HDL, VLDL, and triglycerides, showing no significant difference between the groups.

After the intervention, the probiotic group showed an average decrease of 41.5 mg/dl in serum total cholesterol levels in relation to basal levels. The control group showed a slight increase (8.0 mg/dl) when



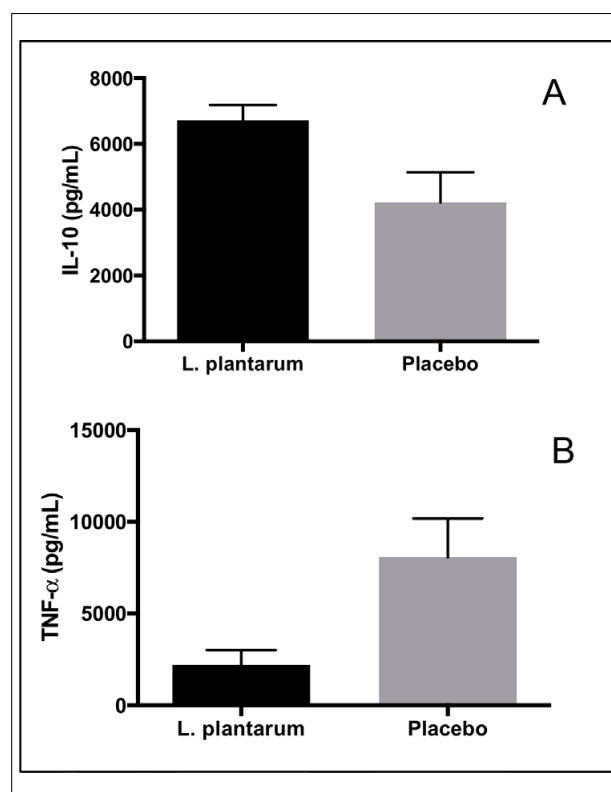
**TABLE 1.** PRE-INTERVENTION BASELINE VALUES IN THE PROBIOTIC AND CONTROL GROUPS.

| Features                   | Probiotic (n=2) mean (DP) | Control (n=2) mean (DP) | P-value* |
|----------------------------|---------------------------|-------------------------|----------|
| Total cholesterol (mg/dl), | 206,5 (2,12)              | 198,0 (1,41)            | 0,121    |
| Total HDL (mg/dl)          | 63,0 (29,69)              | 51,5 (6,36)             | 0,921    |
| VLDL (mg/dl)               | 15,3 (1,32)               | 27,4 (23,75)            | 1,000    |
| LDL (mg/dl)                | 128,2 (28,84)             | 118,0 (27,15)           | 0,439    |
| Triglycerides (mg/dl)      | 76,5 (6,36)               | 137,0 (118,79)          | 1,000    |

Significance value\*  $p \leq 0,05$ .**FIGURE 1.** VARIATION IN A) TOTAL CHOLESTEROL ( $P = 0.439$ ), B) TRIGLYCERIDES ( $P = 0.121$ ), AND C) HDL ( $P = 0.121$ ) LEVELS IN THE PROBIOTIC AND PLACEBO GROUPS (MANN-WHITNEY'S TEST).

compared to normal serum levels. Moreover, in the probiotic group, serum triglyceride levels decreased by 6.0 mg/dl from baseline, while in the control group there was an increase of 49.5 mg/dl. However, serum HDL levels after the intervention decreased by 6.5 mg/dl in the probiotic group when compared to baseline levels and increased by 5.5 mg/dl in the control group (Figure 1). There were no significant changes in weight and waist circumference measurements during the period. Regarding the serum dosages of inflammatory cytokines, the probiotic group presented a TNF- $\alpha$  value about four times lower (2,200.47 pg/ml) than that observed in the control group (8,081.47 pg/ml). IL-10, on the other hand, presented a tendency of increase in the probiotic group of approximately 59% (6,718.55 pg/ml) when compared to the control (4,225.67 pg/ml) (Figure 2).

There were no reports of NS decompensation during the intervention period, nor adverse events or atypical manifestations related to the gastrointestinal tract and systemic. The medicine was checked every 45 days, in order to confirm the proper adherence to treatment.

**FIGURE 2.** VARIATION IN THE LEVELS OF: A) IL-10 ( $P = 0.131$ ) AND B) TNF ( $P = 0.131$ ) IN PROBIOTIC AND PLACEBO GROUPS (MANN-WHITNEY'S TEST).

## DISCUSSION

The preliminary results presented in this study show a tendency of reduction in serum levels of total cholesterol, triglycerides, and TNF- $\alpha$ , and of increase of IL-10, suggesting that *L. Plantarum* probiotic had a satisfactory immunomodulatory and hypolipidemic action in dyslipidemic nephrotic pediatric subjects.

The significant reduction in TNF- $\alpha$  levels observed in this study seemed relevant given the clinical importance of immunomodulation in nephrotic individuals. TNF- $\alpha$  is a proinflammatory cytokine that presents particularly high levels in the acute phase of MCD and FSGS<sup>19</sup>. It stimulates migration, differentiation, proliferation, and cell death. In vascular endothelial cells, these changes induce increased vascular permeability and transendothelial cell migration, favoring atheromatous plaque formation and predisposing to thrombosis<sup>20</sup>. Furthermore, TNF- $\alpha$  also appears to be involved in the podocyte cytoskeleton rearrangement<sup>3</sup>. On the other hand, the use of anti-TNF- $\alpha$  drugs (e.g., etanercept and infliximab) has inexplicably led to NS remission and reduced proteinuria in post-transplanted FSGS children, which serves as clinical evidence of the action<sup>20, 21</sup> of TNF- $\alpha$  in the pathophysiology of proteinuria. This study also found a tendency of increased IL-10 in the probiotic group compared to the control. IL-10 exerts a potent anti-inflammatory effect by inhibiting the production of other interleukins, most notably IL-1 and TNF- $\alpha$ , and the chemokines responsible for the recruitment of macrophages, monocytes, dendritic cells, and T cells<sup>22</sup>. In regard to the participation of the immune system in the triggering of inflammatory complications in NS, the study was useful in demonstrating that the immunomodulatory action that occurred after the use of *L. Plantarum* could probably prove to be a therapeutic option for controlling this inflammation.

Another beneficial effect observed in the probiotic group after the administration of *L. Plantarum* was the reduction in serum total cholesterol and triglyceride levels compared to pre-intervention levels. To date, no scientific literature was found addressing the use of probiotics in dyslipidemic nephrotic individuals. Nonetheless, the present study showed results similar to those observed in different studies that also used *L. Plantarum* in dyslipidemic individuals and reported a satisfactory lipid profile modification<sup>17, 23, 24</sup>.

Limitations regarding the development of the

study need to be addressed, and the sample size is the most relevant. Although the study almost reached the number of individuals calculated to compose the sample ( $n = 12$ ), two factors were determinant in the exclusion of these individuals: first the disagreement between the results of examinations from laboratories belonging to the public health network, and the ones provided by the reference laboratory for biochemical analysis. The children invited to participate in the study were submitted to T0 to collect exams in the reference laboratory, which in most cases did not confirm the existence of dyslipidemia. Second, relapse of NS or infections within 3 months prior to the recruitment limited the selection of children between 2 and 6 years, since they are more susceptible to common childhood infections. Both situations contained in the inclusion criteria aimed to rule out the possibility of selection bias. Also, no fecal analyzes were performed during and after the intervention to confirm the presence and viability of *L. Plantarum* in the fecal microbiota.

However, even in the absence of definitive conclusions, the results favorably signaled a significant clinical impact in favor of probiotic use in NS, which reinforced the interest in disclosing these results as preliminary to a clinical trial, as already published in other occasions<sup>20</sup>.

## CONCLUSION

Nephrotic syndrome is still a disease of high morbidity despite currently available treatments. Complications secondary to the occurrence of cardiovascular events and liponephrotoxicity in nephrotic individuals are directly related to the silent and persistent evolution of dyslipidemia and inflammation<sup>25</sup>.

Thus, the immunomodulatory and hypolipidemic effects presented in the study after the use of probiotic *L. Plantarum* in dyslipidemic nephrotic children are modest, but present new possibilities to be explored regarding the impact of intestinal microbiota on kidney disease.

## Author's Contribution

PMF and PSC designed the study. PMF, RVTF, LHSA, VCJQ collected the data. PMF, RVTF, LHSA, VCJQ, and PSC did the literature search. PMF, RVTF, LHSA, VCJQ, and PSC wrote the paper. PMF, RVTF, LHSA, VCJQ, and PSC approved the final version of the case report.

## Financial support

None.

## Conflicts of interest

No conflicts of interest to be declared concerning the publication of this article.

## RESUMO:

O objetivo deste estudo foi avaliar a eficácia da ação do probiótico *Lactobacillus Plantarum* como um agente imunomodulador e hipolipemiante em crianças e adolescentes dislipidêmicos com síndrome nefrótica.

**MÉTODOS:** Este é um estudo randomizado, duplo-cego, controlado com placebo e realizado em pacientes pediátricos com síndrome nefrótica, compensados ou parcialmente compensados e dislipidêmicos passando por acompanhamento ambulatorial. Variações no lipídio sérico e nas citocinas TNF- $\alpha$  (pró-inflamatória) e IL-10 (anti-inflamatória) foram avaliadas. As citocinas foram analisadas por ensaio imunoenzimático (ELISA).

**RESULTADOS:** No grupo probiótico, houve uma tendência de redução dos níveis de TNF- $\alpha$  e de aumento dos níveis de IL-10, quando comparado ao controle. Em relação ao perfil lipídico, houve uma diminuição nos níveis séricos de triglicérides (6,0 mg/dL) e colesterol total (41,5 mg/dL) no grupo probiótico em comparação aos níveis basais, enquanto no grupo de controle houve um aumento nos níveis séricos de triglicérides (49,5 mg/dL) e colesterol total (8,0 mg/dL).

**CONCLUSÃO:** Os resultados preliminares sugerem que o *L. Plantarum* tem um efeito imunomodulador e hipolipemiante em pacientes pediátricos dislipidêmicos e com síndrome nefrótica.

**PALAVRAS-CHAVE:** Criança. Inflamação. Probióticos. Hipercolesterolemia. Síndrome Nefrótica.

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# Appendectomy: prognostic factors in the brazilian unified health system

 Marcel Gutierrez<sup>1</sup>  
 Thiago Artioli<sup>1</sup>  
 Fábio Iazzetti Lopes<sup>1</sup>  
 Filipe Ramos Monteiro<sup>1</sup>  
 Sandra Di Felice Boratto<sup>1</sup>

1. Centro Universitário Saúde ABC, Santo André, SP, Brasil

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## SUMMARY

**INTRODUCTION:** Acute appendicitis (AA) is the most common cause of surgical acute abdomen. Postoperative complications in emergency care are reflections of the surgical procedure and pre- and postoperative factors.

**OBJECTIVE:** Define prognostic factors for patients who underwent appendectomy, comparing them with the literature.

**METHODS:** Descriptive observational study with a cross-sectional design based on data from the emergency/urgency appendectomy records between September 2018 and April 2019. Variables of interest were considered based on intrinsic patient data, clinical status, and perioperative management factors. Primary outcomes considered: postoperative complications from hospital admission discharge and prolonged hospital stay for > 2 days. Secondary outcome: death. The results were evaluated by Fisher's exact test ( $p < 0.05$ ).

**RESULTS:** We identified 48 patients undergoing an appendectomy. Young adults accounted for 68.7%. From the total, 58.3% were males, 6 (12.5%) had hospitalization > 2 days, 4 (8.3%) had complications and no deaths. Among the variables, the stage of AA, the time of complaint up until seeking care, and advanced age were correlated with worse prognosis during hospitalization ( $p < 0.05$ ). The emergence of immediate postoperative complications was correlated with longer hospital stay ( $p < 0.05$ ).

**DISCUSSION:** The descriptive data of the sample converge with the epidemiological profile of patients with AA in the literature, corroborating the applicability of conventional guidelines. The results strengthen the hypothesis that the patient's flow with abdominal manifestations is complicated from the first contact with SUS to the resolution of the condition.

**CONCLUSION:** Knowledge of the epidemiological profile and perioperative predictors that are most related to complications favor the appropriate management of patients.

**KEYWORDS:** Appendectomy. Unified Health System. Postoperative complications. Prognosis. Health profile.

## INTRODUCTION

Appendectomy occupies a prominent place among surgeries, representing approximately 20% of all surgical interventions, and acute appendicitis (AA) is the most common cause for acute (surgical) abdomen (prevalence 7-10%)<sup>1</sup>. Although it is a condition that

may affect all age groups, AA has a higher incidence among young adults<sup>2</sup>.

The classic symptoms of AA are present in 60% of the cases<sup>2</sup>. Guidelines establish surgery as the gold standard for the treatment of AA<sup>3</sup>, and mortality after

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CORRESPONDING AUTHOR: Thiago Artioli

Avenida Lauro Gomes, 2000, Bairro Vila Sacadura Cabral, Santo André, SP, Brasil – 09060-650

Tel: +55 11 94108-2525

E-mail: thiago.artioli48@gmail.com

appendectomy is low (0,7-4%). Some of the complications most frequently found in the postoperative period are wound infections, wall abscesses, and peritonitis<sup>2</sup>. With proper management, there was a dramatic reduction in mortality attributed to AA over the last 50 years; however, morbidity, which has a strong impact on health care costs, did not present a similar decrease<sup>4</sup>, which is partially explained by the presence of postoperative complications, something that maintains its incidence, particularly in places of lower socioeconomic level<sup>5</sup>. Identifying predictors for postoperative complications in appendectomies is essential to reduce its morbidity. Postoperative complications should be considered when choosing the most adequate management; however, few studies have described relevant predictors to these complications.<sup>6</sup>

Postoperative complications in emergency AA surgeries are a reflection of the surgery itself, and of perioperative factors. Studies suggest that biological sex, presence of perforation, drainage of the abdominal cavity, stage of AA, and time until the completion of surgery are possible determinants for postoperative complications after an appendectomy<sup>7</sup>. However, there are limitations: the lack of studies on prognostic factors, particularly in populations of local services; ignorance about factors that have not yet been discovered; and divergences between existing studies regarding some predictors.

This analysis aims to identify prognostic factors for postoperative complications up until hospital discharge after appendectomies in an emergency care service of General Surgery at the Single Health System.

## METHODS

### Location

This study was conducted on a service linked to the Faculty of Medicine of ABC (FMABC), a reference urgency/emergency center in the region for Emergency Treatments of General Surgery of the Unified Health System (SUS): Municipal Hospital Center of Santo André (CHMSA) - Av. João Ramalho, 326 - Vila Assunção, Santo André - SP, CEP 09030-320.

### Study design

An observational and cross-sectional study that sought to establish, after formal authorization by the clinical board of the Municipal Hospital Center of Santo André (CHMSA) and approval by the local ethics committee, a database of data from the

medical records of patients undergoing emergency/urgency appendectomy between September 2018 and April 2019.

### Population

The sample comprised patients between 5 (five) and 60 (60) years of age who underwent an appendectomy in a context of emergency or urgency, whose necessary information was recorded. Were excluded patients younger than 5 years or older than 60 years, and those who did not have data relating to the variables of interest to this work, as described below.

### Retrieval of the data and definition of variables

The physical records were requested. The variables chosen to determine the demographic profile were age, biological sex, and presence of comorbidities (diabetes), and were selected based on scores for the diagnosis of AA available in the literature<sup>8</sup>.

The variables chosen to determine possible prognostic factors after appendectomy included a review of the literature, which defined analysis of the leukocyte count, duration of symptoms, time of admission to hospital until surgery, perioperative antibiotic therapy, the use of postoperative drain, the reintroduction of diet, and stage of appendicitis<sup>9</sup>.

The combined primary outcomes considered in this study were in-hospital postoperative complications up until hospital discharge and/or extension of the length of hospital stay by over two days. The secondary outcome considered was death up until hospital discharge during the postoperative hospital stay. The complications considered were the most frequently described in the literature: infection of the operative wound, evisceration, the need for patient referral to the ICU in the same hospitalization, intracavitary abscess, and prolonged ileus (defined as fasting for more than 24 hours after the end of the surgery)<sup>7</sup>.

### Informed Consent Form

Informed consent did not apply because the study was conducted based on database analysis.

### Statistical analysis

The data collected were recorded in a Excel (Microsoft) spreadsheet. For the descriptive analysis of quantitative variables that had a normal distribution (Shapiro-Wilk test,  $p > 0.05$ ), we used mean and standard deviation and compared them by Student's *t*-test;



for non-normal distribution, we used median and inter-quartile intervals, compared by the Mann-Whitney test. Categorical variables were expressed by numbers and percentages (frequencies) and were compared by Fisher's exact test. The confidence interval used was 95% and the level of significance was  $p < 0.05$ . The statistical software used was the Data Analysis and Statistical Software for Professionals (Stata) version 13.0.

## RESULTS

We included 48 patients who underwent appendectomy during the period of the study. Male patients accounted for 58.3% of the sample; 29.2% of the patients were aged less than 21 years; 27.1% between 22 and 29 years; 20.8% between 30 and 39 years; 20.8% between 40 and 59 years; and only 2.1% above 60 years. Only one patient (2.1%) had diabetes mellitus.

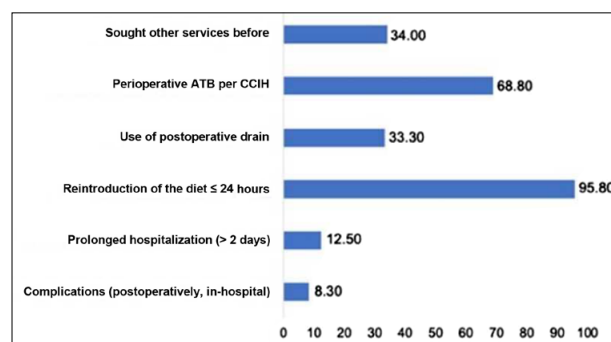
Regarding the perioperative data (Table 1), 68.8% of the cases presented a complaint time of up to two days up to the time of hospitalization, and 66.6% had leukocytosis over 11,000 cells/mm<sup>3</sup>. The time between hospital admission and completion of surgery was 6 to 12 hours in 54.2% of the cases, followed by a time of >12 hours (25%) and, finally, of a time of up to six hours of waiting (20.8%). Other perioperative data can be seen in Figure 1.

**TABLE 1.** CHARACTERISTICS OF PERIOPERATIVE FACTORS

| Variable  | N  | %    |
|---|----|------|
| Time of complaint                               |    |      |
| Up to 2 days                                    | 33 | 68.8 |
| From 2 to 5 days                                | 13 | 27.1 |
| Over 5 days                                     | 2  | 4.2  |
| Total leukocyte                                 |    |      |
| Leukopenia (<4,500/mm <sup>3</sup> )            | 0  | 0    |
| Normal values (4,500 - 11,000/mm <sup>3</sup> ) | 7  | 14.6 |
| Leukocytosis (>11,000/mm <sup>3</sup> )         | 32 | 66.6 |
| Not collected                                   | 9  | 18.8 |
| Time between admission and surgery              |    |      |
| Up to 6 hours                                   | 10 | 20.8 |
| From 6 to 12 hours                              | 26 | 54.2 |
| Over 12 hours                                   | 12 | 25   |
| Appendicitis stage classification               |    |      |
| I   | 12 | 25   |
| II  | 16 | 33.3 |
| III   | 12 | 25   |
| IV  | 8  | 16.7 |
| N Total   | 48 | 100  |

Source: Data from the study.

**FIGURE 1.** PERCENTAGE OF PATIENTS WHO HAD A PERIOPERATIVE FACTOR OBSERVED (MARCEL GUTIERREZ ET AL)



With regards to the perioperative variables studied, the time of complaint up until seeking medical care was correlated with a worse prognosis in comparison to prolonged hospitalization (Fisher's exact test,  $p = 0.046$ ) and in comparison with the presence of complications (Fisher's exact test,  $p = 0.008$ ). The presentation stage of acute appendicitis recorded in the surgical description could also be correlated to an increase in the presence of complications (Fisher's exact test,  $p = 0.012$ ) and increased time of hospitalization after surgery (Fisher's exact test,  $p < 0.001$ ). The presence of postoperative and in-hospital complications was directly proportional to a prolonged hospitalization (Fisher's exact test,  $p < 0.001$ ).

In addition to the relationship between these perioperative factors and outcomes, advanced age was associated with a worse prognosis in comparison to the onset of complications (Mann-Whitney test,  $p = 0.033$  / Fisher test,  $p = 0.103$ ) and prolonged hospitalization (Mann-Whitney test,  $p = 0.0027$  / Fisher test,  $p = 0.03$ ). The correlations found are presented in Table 2.

## DISCUSSION

The study population converges with the epidemiological profile of patients with AA described in the literature<sup>4</sup>. In the study by Lima et al.<sup>10</sup>, also carried out in the context of the SUS, males accounted for 65.2% of the population, while young adults accounted for 60.03%. The rare presence of comorbidities, diabetes mellitus being the main one, was also consistent with the literature<sup>9</sup>.

We observed three main findings. First, it is possible to say that the greater the time of patient complaint at the time of hospitalization, the worse the prognosis in relation to prolonged hospitalization and

**TABLE 2.** DISTRIBUTION OF OUTCOMES IN RELATION TO CORRELATED PROGNOSTIC FACTORS

| Variable                          | Hospital stay<br>> 2 days N (%) | P-value | Presence of<br>complications N (%) | P-value |
|-----------------------------------|---------------------------------|---------|------------------------------------|---------|
| Time of complaint                 |                                 |         |                                    |         |
| Up to 2 days                      | 2 (33.3)                        | 0.046   | 0 (0)                              | 0.008   |
| From 2 to 5 days                  | 3 (50)                          |         | 3 (75)                             |         |
| Over 5 days                       | 1 (16.7)                        |         | 1 (25)                             |         |
| Appendicitis stage classification |                                 |         |                                    |         |
| I                                 | 0 (0)                           | <0.001  | 0 (0)                              | 0.019   |
| II                                | 0 (0)                           |         | 0 (0)                              |         |
| III                               | 1 (16.7)                        |         | 1 (25)                             |         |
| IV                                | 5 (83.3)                        |         | 3 (75)                             |         |
| Age (years)                       |                                 |         |                                    |         |
| < 21                              | 0 (0)                           | 0.03    | 0 (0)                              | 0.103   |
| 22 - 29                           | 1 (16.7)                        |         | 1 (25)                             |         |
| 30 - 39                           | 0 (0)                           |         | 0 (0)                              |         |
| 40 - 59                           | 5 (83.3)                        |         | 3 (75)                             |         |
| > 60                              | 0 (0)                           |         | 0 (0)                              |         |

Source: Data from the study.

the presence of complications. Likewise, the greater the classification of acute appendicitis presentation stage in the surgical description and the greater the age of the patient, also the greater the chances of complications and increased time of hospitalization after surgery.

Findings on the AA stage and the age of patients corroborate previous studies that evaluate prognostic factors in the perioperative period after appendectomies<sup>7</sup>. The AA stage, in more advanced stages, indicates a possible more severe state of the organ, which may present an abscess, necrosis or perforation, favoring the onset of postoperative complications and prolonged hospitalization<sup>10</sup>.

Regarding age, patients of more advanced age are more prone to comorbidities and other conditions that may justify the onset of complications and increased time of hospitalization<sup>6</sup>. However, the comorbidity with a greater correlation with worse prognoses in the literature was diabetes mellitus, and in our sample, it was not possible to demonstrate the influence of this comorbidity on the prognosis. Since our data demonstrated a significant correlation between advanced age and prolonged hospital stay but a weak correlation between advanced age and presence of complications, it is speculated whether age alone would be a factor to keep the patient hospitalized for longer due to a concern of the health team regarding other comorbidities not investigated in this study. Advanced age is also described as being correlated with more atypical

presentations of symptoms, which can influence late diagnosis, increase cases of complicated appendicitis, and increase the time of hospitalization<sup>10</sup>.

The relationship between time of the complaint and prolonged hospital stay and the presence of complications deserves to be highlighted, since, despite the significant correlation in our study, it is a factor that presents many disagreements in the literature. While the study by Andert et al.<sup>11</sup> did not find any correlation between the time from the onset of symptoms to admission with the outcomes, the study by Kim et al.<sup>12</sup> found a relationship between the time and both the presence of complications and the risk of appendix perforation. Other studies prove this latter finding<sup>2</sup>. This fact can be explained, since, theoretically, a greater time of complaint favors the development of the inflammatory process, highlighting the simple conduct that seems to be the fast and accurate diagnosis of AA and the immediate referral to an emergency appendectomy.

The mortality rate in our study was zero, smaller than the indices reported in the literature<sup>9</sup>.

Some limitations present in our study are: reduced patient sample size when compared with similar studies mentioned herein; cross-sectional and observational nature of the study, allowing to establish correlations but not necessarily a causal link between the data; and the presence of bias and confounders, other variables that could not be controlled due to the observational nature of the study.



## CONCLUSION

The AA presentation stage, time of complaint up until seeking assistance, and advanced age are considered prognostic factors for appendectomy because they are correlated with a worse prognosis during the hospital stay, increasing the length of hospitalization and the presence of postoperative complications.

## Author's Contribution

All authors participated in the design, execution, data collection, and analysis of this study and approved the final version. The authors have confirmed that they have no conflicts of interest related to this study, nor financial interests.

## RESUMO

**INTRODUÇÃO:** Apendicite aguda (AA) é causa mais comum de abdome agudo cirúrgico. Complicações pós-operatórias na emergência são reflexos do ato cirúrgico e fatores pré e pós-operatórios.

**OBJETIVO:** Definir fatores prognósticos para paciente pós apendicectomia, comparando com a literatura.

**MÉTODOS:** Estudo observacional descritivo e analítico com formação de base transversal de dados dos prontuários de apendicectomias de emergência/urgência entre setembro de 2018 e abril de 2019. Variáveis de interesse foram consideradas a partir dos dados intrínsecos ao paciente, quadro clínico e manejo perioperatório. Desfechos primários combinados considerados foram complicações pós-operatórias da internação até alta hospitalar e prolongamento da internação > dois dias. Desfecho secundário foi óbito. Resultados avaliados pelo teste exato de Fisher ( $p < 0,05$ ).

**RESULTADOS:** Identificaram-se 48 pacientes submetidos à apendicectomia. Adultos jovens corresponderam a 68,7%. Do total, sexo masculino (58,3%), seis (12,5%) tiveram internação > dois dias, quatro (8,3%) cursaram com complicação e nenhum óbito. Dentre as variáveis consideradas, fase de apresentação da AA tempo de queixa até procura do atendimento e idade avançada correlacionaram com pior prognóstico durante internação ( $p < 0,05$ ). Surgimento de complicações pós-operatórias imediatas correlacionou-se com maior tempo de internação ( $p < 0,05$ ).

**DISCUSSÃO:** Dados descritivos da amostra convergem com perfil epidemiológico de pacientes com AA na literatura, corroborando aplicabilidade das diretrizes convencionais. Resultados fortalecem a hipótese de que o fluxo do paciente com manifestações abdominais é complicado desde o primeiro contato com o SUS até a resolução do quadro.

**CONCLUSÃO:** Conhecimento do perfil epidemiológico e dos preditores perioperatórios que mais se relacionam com complicações favorecem manejo adequado dos pacientes.

**PALAVRAS-CHAVE:** Apendicectomia. SUS. Complicações pós-operatórias. Prognóstico. Perfil de saúde.

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# Development of personalized molds for neovagina creation by 3D printer

 Thyeres Teixeira Bueno Chrispin<sup>1</sup>  
 Marina Silva Fernandez<sup>2</sup>  
 Claudia Cristina Takano Nova<sup>3</sup>  
 Marair Gracio Ferreira Sartori<sup>4</sup>

1. Pós-graduando em Ginecologia – Escola Paulista de Medicina – Universidade Federal de São Paulo (Unifesp) – São Paulo, SP, Brasil.

2. Pós-graduanda em Uroginecologia – Escola Paulista de Medicina – Universidade Federal de São Paulo (Unifesp) – São Paulo, SP, Brasil.

3. Doutora em Ginecologia e Obstetrícia Escola Paulista de Medicina – Universidade Federal de São Paulo (Unifesp) – São Paulo, SP, Brasil.

4. Professora Associada do Departamento de Ginecologia da Escola Paulista de Medicina – Universidade Federal de São Paulo (Unifesp) – São Paulo, SP, Brasil.

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## SUMMARY

Additive Manufacturing (AM), also known as Rapid Prototyping, is a set of production technologies used in the synthesis of a particular physical object by adding layers to form a part based on data generated by Computer-Aided Design (CAD) systems. These technologies are widely used to quickly create prototypes of products and tools for commercial purposes. Over time, it has also been integrated with other areas, such as healthcare, since these tools have allowed health professionals to assist in diagnoses, surgical planning, and synthesis of orthoses and prostheses for patient rehabilitation.

**OBJECTIVE:** To develop models for the construction of dilators for the treatment of vaginal agenesis.

**METHODS:** Use CAD software and create a physical model using AM to analyze the viability of its production in the elaboration of customized dilators for each patient.

**RESULTS:** The production through AM provides an advantage in the development, facilitating physical alterations just by adjusting the three-dimensional models made by the software in a quick way, thus making the customization process viable.

**CONCLUSION:** The proposed procedure for the manufacture of dilators presented good results and technological feasibility, indicating that it can be a good solution for the production and customization of gynecological devices.

**KEYWORDS:** Vagina/abnormalities. Gynecology. Printing, Three-Dimensional. Dilatation/instrumentation.

## INTRODUCTION

### Vaginal agenesis

Vaginal agenesis is a congenital malformation of the reproductive tract of women caused by an anomaly in the formation of the paramesonephric ducts (Müller), responsible for originating the internal genital organs in females<sup>1,2</sup>.

Most vaginal agenesis cases are related to the Mayer-Rokitansky-Kuster-Hauser (MRKH) syndrome,

characterized by complete müllerian agenesis. Its incidence ranges from 1 for every 4,000/5,000 live births of females, and its identified by the congenital absence of the uterus and the superior  $\frac{2}{3}$  of the vagina<sup>1</sup>.

The diagnosis can be made by a simple physical examination, but it can be evidenced since birth by the examination of the genitals. Usually, the

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CORRESPONDING AUTHOR: Thyeres Teixeira Bueno Chrispin

Departamento de Ginecologia – Unifesp – Rua Napoleão de Barros, 638, Vila Clementino, São Paulo, SP, Brasil – 04024-002

Tel: +55 11 98436-4366

E-mail: thyeres.unifesp@gmail.com

diagnosis is established during adolescence, when the patient seeks assistance due to complaints of absent menstruation and/or difficulty in sexual intercourse<sup>3</sup>.

Faced with these facts, a multidisciplinary approach is required from the beginning of the treatment, with a joint action of medical, psychological, nursing, technological, and physical therapy teams<sup>3</sup>.

Despite the rare incidence of this condition, its treatment has been widely discussed and studied. Currently, the conservative method has been considered the first-line treatment, for obvious reasons (low rate of complications, ease of implementation, low cost). This treatment is based on creating a vaginal canal neoformed from the progressive dilation of the vaginal introitus using acrylic dilators. This method was developed by Frank in 1938, and modified by Ingram in 1981<sup>4,5</sup>.

In 2013, the American Congress of Gynecology and Obstetrics recommended vaginal dilation as the first-line treatment for vaginal agenesis. This technique also is the technique of choice in several other countries such as the United Kingdom, Australia, the United States, and Russia. Vaginal dilation as the first-line treatment for vaginal agenesis has the benefit of being a safer technique, in addition to presenting a significant success rate<sup>6</sup>.

The main purpose of the vaginal dilators is to promote the dilation and awareness of the pelvic floor muscles in women with any dysfunction in this area. These devices vary in size and thickness, something important for the treatment, which is progressive in nature<sup>7</sup>.

Kits sold commercially, in general, contain six penetrators made of silicone, their surface is smooth and their sizes vary from 1.2 cm to 4 cm in diameter and from 6.5 cm to 14.5 cm in length; however, devices made of silicone are not fitting for vaginal agenesis cases because they are too flexible. The more rigid models are not sold in Brasil.

Typically, these dilators come in specific and standardized lengths and diameters, which may hinder their use in different patients, making it necessary to have a custom-made model, on a case by case basis.

AM, better known as 3D printing, is a manufacturing process based on the creation of a physical object by adding layers of a certain material, using data generated by a mechanical design software that uses CAD (Computer-Aided Design) technology<sup>7-9</sup>.

These technologies are widely used for quickly

creating prototypes, products, and tools for commercial purposes<sup>10</sup>.

The most common and widely known 3D printers have as their basic physical principle the fusion and extrusion of thermoplastic materials and deposition of such material in layers until the desired object is formed. This method is known as FDM (Fused Deposition Modeling)<sup>11</sup>.

A characteristic feature of 3D printers is their ability to create an object using a digital drawing in a type of software capable of reading the entire surface of the object drawn. The most widely known file format for reading CAD drawings is STL (Stereo Lithography)<sup>9</sup>.

Among the health areas in which MA is used most prominently, currently, are surgical aid tools, tools for aiding physicians in exams or procedures, orthopedic implants and prostheses. This field also presents great research possibilities regarding the creation of new technologies and, consequently, a breakthrough in healthcare<sup>12</sup>.

Particularly among women with vaginal agenesis, the creation of custom vaginal dilators can be an excellent alternative treatment. Each patient could benefit from the creation of dilators in custom sizes and formats to fit their anatomy. Thus, based on the evolution of treatment, new Dilators could be designed according to the needs of each patient<sup>13</sup>.

Therefore, AM could contribute to the development of these devices, meeting the specific needs of each patient and providing more individualized care.

## OBJECTIVE

Prepare digital models for building custom dilators for the treatment of vaginal agenesis by additive manufacturing.

## METHODS

The preparation of the procedure for the development of the devices was divided into five steps:

### 1. Survey of the requirements and assessment of the parameters of use

In order to specify the requirements for the development of a prototype, we conducted a survey with gynecology professionals and on the devices already on the market to obtain information on the characteristics, dimensions, and parameters (ergonomic and geometric) required to produce the dilators.

## 2. Development of the model using a CAD software

The modeling of the prototypes was done using the 3D parametric modeling software AutoCAD and FreeCAD based on the requirements previously defined.

## 3. Manufacturing of the dilators by Additive Manufacturing

The production of the devices was done using the 3D printer Cube, developed by the company 3D Systems, using polymeric polylactic acid (PLA) filaments.

## 4. Analysis of the quality of the dilators produced

After the device was manufactured by the 3D printer, the piece went through a quality check to verify its mechanical resistance, geometrical structure and whether the physical object produced had the characteristics determined during the modeling step.

## 5. Analysis of the devices by a team of professionals

To check if the prototype produced met all the requirements established, the device underwent an evaluation by three specialists in the area, who analyzed all aspects and assessed whether the device adequately met the defined needs. The experts were gynecologists from the Genital Malformation Unit of the Department of Gynecology of the *Escola Paulista de Medicina*, UNIFESP.

## RESULTS

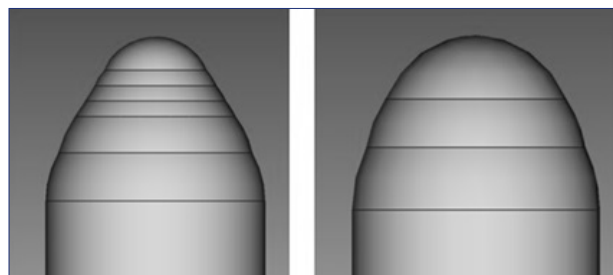
### Survey of the requirements

Based on the analysis of products with similar systems and by carrying out discussion sessions with the members of the project and experts, three models of non-flexible dilators were established, measuring 12.0 cm, 10.0 cm, and 8.0 cm in height and 2.5 cm, 2.0 cm, and 1.5 cm in diameter, respectively, in addition to determining the ergonomic and geometric conditions for the devices, always taking into account their ability to be customized.

After the first model was developed and a discussion was carried out to ascertain whether the device met the specifications proposed by the professionals, it was concluded that changes on the top surface of

the device were necessary to make it more rounded. These changes were carried out, and the device was taken again for evaluation by the group of professionals, who found that the modified device met the proposed specifications.

**FIGURE 1.** THREE-DIMENSIONAL MODELS DESIGNED USING MODELING SOFTWARE A

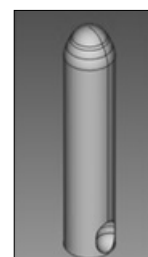


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There modeling step in the CAD environment was adjusted according to the parameters collected from the previous model of the device that was commercially available. The data observed during the explanation of the use of the device substantially influenced the improvement of the geometric characteristics of the final three-dimensional model.

The software-based modeling produced designs that met the needs for the production of the device and the fundamental characteristics for it to perform the specific functions of the treatment.

**FIGURE 2.** THE FINAL THREE-DIMENSIONAL MODEL OF THE VAGINAL DILATOR



Author: Thyeres T. B. Chrispin

The process of creating the devices by AM was completed as expected, resulting in the production of pieces per the requirements established in the first stage, with a dimensional accuracy of up to 0.2 mm, a variation that does present problems for customization or the treatment.

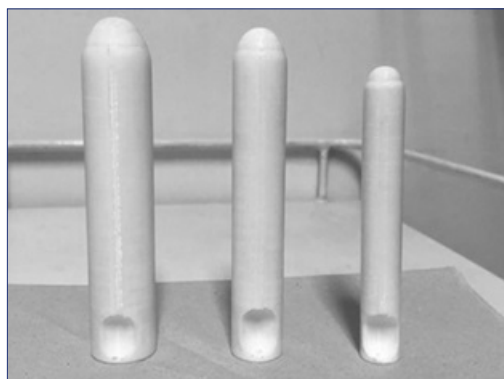
## Quality of the dilators

AM proved to be a viable system for manufacturing dilators. The polymers used showed good mechanical resistance in different settings. The dimensional stability remained constant and the constancy of the dimensional repeatability also presented results as expected.

## Professional opinion

According to the assessment by the professionals, the dilator met the objective proposed without failure, ie., the prototype has all the characteristics to meet the requirements proposed in the first step and that allow it to perform the procedures for the treatment of vaginal agenesis.

**FIGURE 3.** DILATOR MODELS PRODUCED BY 3D PRINTING



Author: Thyeres T. B. Chrispin

## DISCUSSION

The ergonomics, thickness, and length of a vaginal dilator are directly related to the anthropometric measures of its user.

The existing dilators, despite their variation in the INTERROGATORIES length and thickness, probably there would meet all the characteristics of ergonomics are necessary.

During the process of modeling using CAD software, it was possible to produce designs that met the requirements specified by the professionals, and it was proven that the customization of models using the software is perfectly possible, resulting in models that are adequate to the ergonomic characteristics of each person.

The drawings are saved in STL will remain stored in the system of the Department of Gynecology of Unifesp so that, in the future, they can be shared, through an app or via the web, with other health professionals.

Production using AM presented an advantage in the development, making it easier to implement physical changes only by quickly adjusting the 3D models made by software, making the process of customization viable.

The next step in this study is to check with the specialists the effectiveness of the dilators produced by AM, as well as investigate the possibility of custom care by using this technology.

## CONCLUSION

The proposed procedure for the manufacturing of the dilators showed good results and technological feasibility, indicating that it may be a good solution for the production and customization of gynecological devices.

AM technology by thermofusion and its accuracy have met the specific needs of the study. Now, new studies are required related to its feasibility in treatments with monitoring by professionals in the area.

## RESUMO

A Manufatura Aditiva (MA), também conhecida como Prototipagem Rápida, é um conjunto de tecnologias de produção utilizado na síntese de determinado objeto físico por meio da adição de camadas para formar uma peça com base em dados gerados por sistemas de projeto auxiliado por computador (CAD - Computer Aided Design). Essas tecnologias são muito utilizadas para criação de protótipos de produtos e ferramentas de maneira rápida para fins comerciais. Com o tempo, também se integrou a outras áreas, como a área da saúde, uma vez que essas ferramentas permitiram auxiliar o profissional da saúde em diagnósticos, planejamento cirúrgico e na síntese de órteses e próteses para reabilitação de pacientes.

**OBJETIVO:** Elaborar modelos computadorizados para a construção de dilatadores para tratamento de agenesia vaginal pela impressão 3D.

**MÉTODO:** Utilização de software CAD e criação do modelo físico por meio de MA para análise da viabilidade de sua produção na elaboração de dilatadores customizados para cada paciente.

**RESULTADOS:** A produção por meio de MA atribui uma vantagem ao desenvolvimento, facilitando as alterações físicas apenas ajustando os modelos tridimensionais feitos pelos softwares de maneira rápida, tornando o processo de customização viável.

**CONCLUSÃO:** O procedimento proposto para a fabricação dos dilatadores apresentou bons resultados e viabilidade tecnológica, indicando que pode ser uma boa solução de produção e customização de dispositivos ginecológicos.

**PALAVRAS-CHAVE:** Vagina/anormalidades. Ginecologia. Impressão tridimensional. Dilatação/instrumentação.

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# Effects of nasal aspiration by the Proetz® method in pediatric patients with sinusitis

 Yaskara Amorim Filgueira<sup>1</sup>  
 Vanderlan Nogueira Holanda<sup>2</sup>  
 Fernando Luiz Affonso Fonseca<sup>3</sup>  
 David Feder<sup>3</sup>

1. Departamento de Fisioterapia, Centro Universitário Doutor Leão Sampaio – Unileão, Juazeiro do Norte, CE, Brasil.

2. Departamento de Bioquímica, Centro de Biociências, Universidade Federal de Pernambuco, Recife, Brasil.

3. Centro Universitário Saúde ABC, Santo André, SP, Brasil.

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## SUMMARY

**OBJECTIVE:** To characterize the effects of nasal aspiration with Proetz® in peak nasal inspiratory flow (PNIF) in pediatric sinusitis (PS) patients with nasal obstruction.

**METHODS:** This is a non-randomized descriptive-analytical clinical trial with a quantitative approach. The sample comprised 30 children. Initially, the PNIF was measured and the Visual Analogical Scale (VAS) was used for nasal obstruction, followed by the nasal aspiration procedure. The SNOT-22 questionnaire was applied to the legal guardian of each child, and one week later, it was reapplied for the sake of follow-up.

**RESULTS:** 16 (53.3%) patients were females and 14 (46.7%) were males, with an average age of 6.4±1.8 years (between 4 and 10 years of age). Analyses of the VAS for obstruction before the intervention revealed that 10 of the participants (33.3%) presented moderate levels, and 20 of them (66.7%) severe levels. However, after the Proetz® method was applied, all the samples (n=30) had mild levels. The PNIF significantly increased after the technique was used, with an improvement of 23.4% in mean values. There was no significant correlation between the VAS and the PNIF.

**CONCLUSION:** Nasal aspiration with the Proetz® method significantly improved the clinical condition of sinusitis patients with nasal obstruction according to the visual analogical scale, the PNIF, and the SNOT-22 questionnaire. No correlation between the VAS and the PNIF could be found. The study confirms the importance of non-pharmacological interventions in the treatment of sinusitis in children, thus resulting in an improvement in their quality of life.

**KEYWORDS:** Sinusitis. Respiratory Tract Infections. Child. Nasal Obstruction.

## INTRODUCTION

Nasal obstruction is a common symptom of upper-airway disorders in children, and conservative therapy for nasal passage obstruction, whenever indicated, is essential<sup>1</sup>. However, to reestablish the drainage pathway by clearing the paranasal sinuses is as important as eliminating the etiologic agent<sup>2</sup>. Some

interference of nasal cleansing and massage over nasal aeration can be observed in children with physiological mouth breathing. Nasal aeration measures showed sensitivity to the cleansing and massage techniques, and measures of nasal geometry confirmed its effect on respiratory physiology<sup>3</sup>.

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CORRESPONDING AUTHOR: Yaskara Filgueira

Av. Maria Letícia Leite Pereira, s/n, Juazeiro do Norte, CE, Brasil – 63022-145

Tel: +55 88 98812-5774

E-mail: yascarafilgueira@leaosampaio.edu.br

In order to relieve the nasal obstruction in the upper airways, some techniques lead to good results, like the nasal instillation of saline solution<sup>4</sup>, therapeutic ultrasound<sup>5</sup> and nasal aspiration with Proetz®<sup>6</sup>. The method of nasal aspiration with Proetz® was described by and named after Arthur W. Proetz in 1926<sup>7</sup>. It consists of the clearing of the nasal cavity to decongest the upper airways. This technique is performed through the nostrils by means of suction that exerts negative pressure in the nasal cavities. It is based on the air elasticity principle in the paranasal cavities and the gas-compressibility law for the displacement of secretions<sup>8</sup>.

It is known that PA usually causes from 6 to 8 upper airway infectious attacks a year, with high symptomatology of nasal duct obstruction<sup>9</sup>. Therefore, studies on nasal aspiration with the Proetz® method are justified, and if proven effective, it may open doors to the pediatric society to a new therapeutic method based on scientific evidence regarding the efficacy of the technique in the treatment of nasal obstruction.

The guiding question of this study was designed based on the PICO acronym, in which P stands for the Population (pediatric patients - PA), I for Intervention (effects of nasal aspiration with Proetz® method), and CO for Context (nasal obstruction due to sinusitis diagnosis). Therefore, the following question arose: Is the Proetz® method effective for the removal of secretions from the nasal duct in pediatric patients?

The aim of this study was to characterize the effects of nasal aspiration with Proetz® in PNIF in PS patients with nasal obstruction.

## METHODS

This is a non-randomized descriptive-analytical clinical trial with a quantitative approach. It was conducted at the Respiratory Physiotherapy Department, pediatric wing, from the Centro Universitário Doutor Leão Sampaio (Unileão), in Juazeiro do Norte, Brasil, in May 2017. The study was approved by the Research Ethics Committee of Unileão under n° 2256330. All the parents or guardians signed a term of consent and patients signed an assent term.

The sample comprised 30 male and female children aged between 4 and 10 years. They were selected by intentional sampling, including sinusitis patients with nasal obstruction whose guardians signed all the terms presented to them. The exclusion criteria were: children with neurologic diseases, with cognitive, renal,

metabolic and/or cardiovascular alterations; those with facial trauma, headache, facial burns, recurrent epistaxis, diagnosis of neoplasia; patients in a mediate or immediate postoperative period; children who could not complete the tests presented by the researchers.

The treatment was carried out in the afternoon, in a climate-controlled environment, at the same temperature proposed in the study by Teixeira et al.<sup>10</sup>, namely, between 22 and 24°C. At first, the children were submitted to a clinical examination that included an ectoscopy followed by the measurement of the PNIF and evaluation using the VAS.

The PNIF was measured to analyze how much the nasal obstruction interfered with the intake of air, or if there was no direct influence. The measurement was performed by using an in-check inspiratory flow meter (Clement Clark International) with a padded oronasal mask over the face of the patient. The values of the analysis ranged from 30 to 370 L/min registered on the surface of the cylinder after each inhalation. The result was immediately obtained.

The VAS was carried out to evaluate the level of nasal obstruction according to the child's subjective perception. The scale ranges from 0 to 10, and it is organized by colors that describe the intensity of nasal congestion, which goes from mild to moderate, to severe. This scale has already been used in other studies with good acceptance and promising results for the subjective analysis of pain in children and adolescents, like in the study conducted by Tostes et al.<sup>11</sup>.

It is important to point out that before the measurement of PNIF was performed, it was clearly and concisely explained to the patient, taking each age into account. The patient was placed in a sitting position, and the mask was properly adjusted to the face so that no air could escape from it. To start the procedure, the command "close your mouth and smell the flower very quickly" was given, which, in a playful way, corresponds to quickly breathing in through the nose. Three measurements were taken from each patient at intervals of 1 minute, and at the end, the highest was selected. If a variation higher than 40 L/min was detected between measurements, a new evaluation was done.

Once the measurements were taken and after a resting period, the SNOT-22 questionnaire was applied. Translated into Portuguese by Kosugi et al.<sup>12</sup>, this questionnaire analyzes symptoms during two weeks prior to its application. The questions were adapted so that the legal guardians could answer

them. Although it is self-applicable, the researchers were available to help the respondents, without interfering in their answers, when questions came up.

The SNOT-22 is specific for sino-nasal disorders. It is composed of 22 questions that encompass nasal, paranasal, and psychological symptoms as well as aspects related to the quality of sleep. Each question receives a score from 0 to 5, where 0 is the absence of a condition and 5 is the most severe case of the condition. The questionnaire also evaluates the quality of life of sinusitis patients; therefore, it is also applied whenever interventional processes or therapeutic procedures are to be conducted so that their efficacy and the significant effects on the life of the patient can be analyzed. SNOT-22 is considered the best questionnaire for the evaluation of sinusitis<sup>13</sup>.

After all the analyses were carried out, nasal aspiration with the Proetz® method was performed. The patient was in a quiet environment, lying on a stretcher with no head elevation, and the therapist was alongside the head of the stretcher for the procedure. A 10 ml syringe was used for the gradual introduction of 0.9 saline solution to thin the secretions, and then the vacuum aspiration (-150/-180mmHg) technique was applied. The same process was repeated for the second nostril. The whole procedure lasted from 2 to 4 minutes aiming to remove all the secretions from the paranasal sinuses, and the PNIF could then be reassessed.

The statistical analysis was performed using the Shapiro-Wilk test for the analysis of data normality. It was observed that the variable post-inspiratory pressure did not show normal distribution ( $p=0.011$ ), and the variable post-VAS had a probability value close to non-normal distribution (0.071). Hence, the descriptive values mean, standard deviation, median, and 25-75 percentiles were shown. For inferential comparison, the Wilcoxon and Spearman's rank tests were used for the variables from the SNOT-22 questionnaire. The Stata 11 software was used for data analysis.

## RESULTS

The sample was composed of 30 children clinically diagnosed with sinusitis. There were 16 females (53.3%) and 14 males (46.7%) at an average age of  $6.4 \pm 1.8$  years, ranging between 4 and 10 years ( $S^2$  3.206 and  $SE$  0.327). The means and medians obtained from the VAS and PNIF before and after the Proetz® method are shown in Table 1.

Initially, it could be observed that the VAS scores significantly improved with the Proetz® method. Before the procedure, 10 (33.30%) of the participants had a moderate score, and 20 (66.70%) had a severe score. However, after the method was used, the entire sample ( $n=30$ ) presented a mild score, indicating that there was a reduction in nasal obstruction according to the patients' perception.

The analysis of the PNIF measurement showed significant improvement after the procedure. Before the application of the technique, the values ranged from 40 to 100 L/min. On the other hand, after the intervention, these values ranged from 50 to 120 L/min in all patients, with an increase of 80% in one of them.

**TABLE 1.** DESCRIPTIVE VALUES AND TESTING OF HYPOTHESIS FOR THE DIFFERENCES IN THE VISUAL ANALOGICAL SCALE (VAS) AND INSPIRATORY PRESSURE BEFORE AND AFTER THE PROCEDURE.

| Indicators            | VAS          |              |        | Inspiratory pressure |                 |        |
|-----------------------|--------------|--------------|--------|----------------------|-----------------|--------|
|                       | Before       | After        | p*     | Before               | After           | p*     |
| Mean (sd)             | 7.5<br>(1.4) | 2.6<br>(1.0) | <0.001 | 64.0<br>(20,1)       | 79.8<br>(20,4)  | <0.001 |
| Median<br>(p25 – p75) | 7.0<br>(7–8) | 2<br>(2–3)   |        | 60<br>(50 –80)       | 80<br>(60 –100) |        |

sd = standard deviation; p25 = 25 percentile; p75 = 75 percentile; \* Wilcoxon test.

Table 2 shows the Spearman correlation for VAS and PNIF before and after the Proetz® method. No statistical significance could be found in this correlation.

**TABLE 2.** SPEARMAN CORRELATION BETWEEN VAS BEFORE - INSPIRATORY PRESSURE BEFORE AND VAS AFTER - INSPIRATORY PRESSURE AFTER.

| Correlation                              | Spearman rank correlation | p     |
|--|---------------------------|-------|
| VAS before – inspiratory pressure before | 0.26                      | 0.168 |
| VAS after – inspiratory pressure after   | 0.14                      | 0.465 |

\*VAS: Visual Analogical Scale

The analyses of the SNOT-22 questionnaire can be found in Table 3.

According to the data supplied by the patients, after the procedure, there was a statistical significance for the variables “need to blow nose”, “sneezing”, “runny nose”, “cough”, “post-nasal discharge”, “thick nasal discharge”, “ear fullness”, “ear pain”, “facial pain/pressure”, “difficulty to fall asleep”, “wake up at night”, “reduced concentration”, “frustrated/restless/irritable”, “sad”, “decreased sense of smell/taste” and “nasal blockage”. The results reveal that there is a direct impact on the patients' quality of life.

**TABLE 3.** ANALYSIS OF THE VARIABLES FROM THE SNOT-22 QUESTIONNAIRE (N=30).

|                                | BEFORE    |       |                | AFTER     |       |                | Wilcoxon** |        |
|--------------------------------|-----------|-------|----------------|-----------|-------|----------------|------------|--------|
|                                | $\bar{x}$ | sd    | S <sup>2</sup> | $\bar{x}$ | sd    | S <sup>2</sup> | Z          | p      |
| Need to blow nose              | 3.470     | 0.681 | 0.464          | 0.700     | 0.651 | 0.424          | -4.843     | 0.001  |
| Sneezing                       | 1.400     | 1.303 | 1.697          | 0.400     | 0.498 | 0.248          | -3.159     | 0.002  |
| Runny nose                     | 3.800     | 0.714 | 0.510          | 0.700     | 0.651 | 0.424          | -4.836     | 0.001  |
| Cough                          | 0.730     | 0.785 | 0.616          | 0.430     | 0.504 | 0.254          | -2.065     | 0.039  |
| Post-nasal discharge           | 0.930     | 0.907 | 0.823          | 0.500     | 0.509 | 0.259          | -2.230     | 0.026  |
| Thick nasal discharge          | 3.130     | 1.252 | 1.568          | 0.630     | 0.556 | 0.309          | -4.737     | 0.001  |
| Ear fullness                   | 0.270     | 0.450 | 0.202          | 0.000     | 0.000 | 0.000          | -2.828     | 0.005  |
| Dizziness or vertigo           | 0.000     | 0.000 | 0.000          | 0.000     | 0.000 | 0.000          | 0.000      | 1.000* |
| Ear pain                       | 0.270     | 0.450 | 0.202          | 0.000     | 0.000 | 0.000          | -2.828     | 0.005  |
| Facial pain/pressure           | 2.570     | 0.504 | 0.254          | 0.630     | 0.490 | 0.240          | -4.847     | 0.001  |
| Difficulty to fall asleep      | 2.270     | 0.691 | 0.478          | 0.670     | 0.479 | 0.230          | -4.800     | 0.001  |
| Wake up at night               | 2.330     | 0.661 | 0.437          | 0.430     | 0.504 | 0.254          | -4.700     | 0.001  |
| Lack of a good night's sleep   | 0.530     | 0.730 | 0.533          | 0.230     | 0.430 | 0.185          | -1.726     | 0.084* |
| Wake up tired                  | 0.430     | 0.504 | 0.254          | 0.230     | 0.430 | 0.185          | -1.420     | 0.180* |
| Fatigue                        | 0.430     | 0.504 | 0.254          | 0.230     | 0.430 | 0.185          | -1.342     | 0.180* |
| Reduced productivity           | 0.530     | 0.507 | 0.257          | 0.370     | 0.490 | 0.240          | -1.508     | 0.132* |
| Reduced concentration          | 1.300     | 0.794 | 0.631          | 0.470     | 0.507 | 0.257          | -3.000     | 0.001  |
| Frustrated/restless/irritable  | 2.030     | 0.669 | 0.447          | 0.570     | 0.504 | 0.254          | -4.932     | 0.001  |
| Sad                            | 2.000     | 0.830 | 0.690          | 0.130     | 0.346 | 0.120          | -4.686     | 0.001  |
| Embarrassed                    | 0.000     | 0.000 | 0.000          | 0.000     | 0.000 | 0.000          | 0.000      | 1.000* |
| Decreased sense of smell/taste | 2.030     | 0.809 | 0.654          | 0.470     | 0.507 | 0.257          | -4.421     | 0.001  |
| Nasal blockage                 | 3.230     | 0.898 | 0.806          | 0.700     | 0.651 | 0.424          | -4.90      | 0.001  |

$\bar{x}$  (mean); sd (standard deviation); S<sup>2</sup> (variance); Z (Wilcoxon value); p (significance <0.05);\*Statistically non-significant;\*\* Non-parametric Wilcoxon signed-rank test.

Source: Own authorship

## DISCUSSION

Besides drug therapies and surgical approaches that target the improvement in airway clearance, there are other resources that can bring the breathing function closer to normal. Such resources consist of a clinical procedure called nasal irrigation, which allows for an improvement in bilateral air intake and output through the cleansing of the airways with 0.9 saline and non-invasive aspiration. However, the results obtained from this procedure regarding nasal permeability are usually subjectively evaluated. The quantitative measurements of peak nasal inspiratory and expiratory flow are of utmost importance<sup>14</sup>.

A study conducted by Fritz<sup>15</sup> showed the great efficacy of the Proetz® method in children in the treatment of paranasal sinus obstruction, especially the ethmoid sinus. Such results minimize or eliminate the complications brought by sinusitis in this population. Stancic<sup>16</sup> had a sample of 166 children aged between 6 months and 14 years with upper airway obstructions who did not respond to conventional pharmacological treatment. When the Proetz® method was used, it helped in the patients' full recovery from nasal obstruction conditions.

The result obtained from the use of the Proetz® method regarding the improvement in nasal clearance and permeability increases aeration efficiency due to the clearance of the paranasal sinuses. Therefore, the effect of the method on nasal permeability, in structural terms related to the nasal mucosa, is significant, the functional effect on aeration was a fact that can be considered satisfactory for the recovery from nasal obstructive conditions. Tugrul et al.<sup>17</sup> worked with a sample of 90 children suffering from acute rhinosinusitis on whom the nasal obstruction treatment was performed. There was a significance in the values of PNIF after the procedure. Soler et al.<sup>18</sup> conducted a study with individuals between 2 and 21 years old diagnosed with chronic rhinosinusitis. They underwent conservative treatment for 6 months before and after the intervention. According to the results obtained from the SNOT-22 questionnaire, the patients seemed to be confident regarding their overall quality of life with mean values  $42,2 \pm 19,2$  vs.  $10,4 \pm 9,7$   $p < 0,0001$ .

Ciprandi et al.<sup>19</sup> studied the correlation of VAS for nasal obstruction and the PNIF. The researchers

confirmed the relevance of the clinical use of the scale, with Spearman's coefficient  $\rho=0.879$  ( $p<0.001$ ).

Hence, the application of the method in PA with nasal obstruction is strongly recommended. After the Proetz® method, according to the VAS for nasal obstruction, patients described their pain as mild, and an improvement in nasal obstruction and in nasal aeration could be observed. The insufficient sample size, as well as the lack of comparative literature on the subject (very few similar studies using the same technique in different populations), were factors that posed as limitations to the current study.

## CONCLUSION

It could be observed that nasal aspiration with the Proetz® method significantly improved the clinical condition of sinusitis patients with nasal obstruction

according to the visual analogical scale, the PNIF, and the SNOT-22 questionnaire. No correlation between the VAS and the PNIF could be found. The study confirms the importance of the application of non-pharmacological interventions in the treatment of sinusitis in children, thus resulting in an improvement in their quality of life. Additionally, this group of researchers hopes to enrich the national literature with further knowledge on the real effects of Proetz®, a valuable tool for pediatric physiotherapy.

## Author's Contribution

Yaskara Amorim Filgueira: Conceptualization, Validation, Investigation, and Writing; Vanderlan Nogueira Holanda: Writing - Review & Editing; Fernando Luiz Affonso Fonseca: Investigation, Supervision, Project Management; David Feder: Investigation, Supervision, Writing - Editing, Project Management.

## RESUMO

**OBJETIVO:** Caracterizar os efeitos da aspiração nasal com Proetz® no pico do fluxo inspiratório nasal (Pnif) em pacientes com sinusite pediátrica (SP) com obstrução nasal.

**MÉTODOS:** Trata-se de um ensaio clínico analítico descritivo, não randomizado, com abordagem quantitativa. A amostra foi composta por 30 crianças. Inicialmente, o Pnif foi medido e a escala visual analógica (EVA) foi utilizada para obstrução nasal, seguida do procedimento de aspiração nasal. O questionário Snot-22 foi aplicado ao responsável legal de cada criança e, uma semana depois, foi reaplicado para fins de acompanhamento.

**RESULTADOS:** Dezesesseis (53,3%) pacientes eram do sexo feminino e 14 (46,7%) do sexo masculino, com idade média de  $6,4\pm 1,8$  anos (entre 4 e 10 anos). A análise da EVA para obstrução antes da intervenção revelou que dez dos participantes (33,3%) apresentaram níveis moderados e 20 deles (66,7%), níveis graves. No entanto, após a aplicação do método Proetz®, todas as amostras ( $n=30$ ) apresentaram níveis leves. O Pnif aumentou significativamente após a utilização da técnica, com uma melhoria de 23,4% nos valores médios. Não houve correlação significativa entre EVA e Pnif.

**CONCLUSÃO:** A aspiração nasal com o método Proetz® melhorou significativamente o quadro clínico de pacientes com sinusite com obstrução nasal, de acordo com a escala visual analógica, o Pnif e o questionário Snot-22. Não foi encontrada correlação entre EVA e Pnif. O estudo confirma a importância de intervenções não farmacológicas no tratamento da sinusite em crianças, resultando em melhoria na sua qualidade de vida.

**PALAVRAS-CHAVE:** Sinusite. Infecções respiratórias. Criança. Obstrução nasal.

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# Detection of *Helicobacter pylori* in oral yeasts from students of a Chilean university

 Kimberly Sánchez-Alonzo<sup>1</sup>  
 Cristian Parra-Sepúlveda<sup>1</sup>  
 Lorena Vergara<sup>2</sup>  
 Humberto Bernasconi<sup>3</sup>  
 Apolinaria García-Cancino<sup>1</sup>

<sup>1</sup>. Laboratorio de Patogenicidad Bacteriana, Departamento de Microbiología, Facultad de Ciencias Biológicas, región del Biobío, Chile.  
<sup>2</sup>. Departamento de Odontología Restauradora, Facultad de Odontología. Universidad de Concepción, Concepción, Chile.  
<sup>3</sup>. Laboratorios Pasteur. Concepción, Chile.

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## SUMMARY

**INTRODUCTION:** Nearly 73% of the Chilean population is infected with *Helicobacter pylori* (*H. pylori*), a factor predisposing for gastric cancer. Recent studies have demonstrated the presence of this pathogen within yeasts, suggesting that this fact can directly influence the failure of a treatment, transmission, and reinfection.

**AIM:** To detect the presence of *H. pylori* inside oral yeasts isolated from students of the University of Concepción (Chile).

**METHODS:** 72 samples, obtained from the oral cavity using cotton swabs were incubated in YPD broth for 48h at 37°C and posteriorly seeded in Sabouraud Dextrose agar plus chloramphenicol at the same temperature and for the same time. Yeasts isolated were observed microscopically (wet mounting and Gram-stained) and identified using microbiological techniques. Intracellular *H. pylori* detection was performed by the amplification of 16S rDNA by PCR.

**RESULTS:** Oral yeasts were detected in 24 samples (33.3%), being *C. albicans* (79.2%) the most frequent species, followed by *C. dubliniensis* (12.4%), *C. krusei* (4.2%), and *C. tropicalis* (4.2%). When analyzed by PCR, 15 of the 24 oral yeasts 62.5 % were positive for *H. pylori* 16S rDNA. From the 15 individuals positive for yeast harboring *H. pylori*, 81% of them reported stomach discomfort, and the presence of the bacteria was diagnosed at some moment in 20% of them.

**CONCLUSION:** The intracellular presence of the *H. pylori* in oral yeasts suggests an endosymbiotic relationship of these microorganisms, which could favor *H. pylori* transmission and reinfection in the gastrointestinal tract.

**KEYWORDS:** *Helicobacter pylori*. *Candida*. Mouth.

## INTRODUCTION

*H. pylori* is a Gram-negative bacterium with several virulence factors, which allow it to selectively colonize the gastric mucosa. It is the etiological agent of several gastrointestinal diseases in humans<sup>1-3</sup>. The latter acts as its main reservoir, acquiring an infection mainly during childhood, supposedly in the intrafamily

environment. However, how this pathogen spreads is not fully understood; up until now, it is accepted that its transmission occurs through the oral-oral and fecal-oral route. Although *H. pylori* has been studied for decades, questions remain regarding how it spreads among populations, since *H. pylori* genes have been

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CORRESPONDING AUTHOR: Apolinaria García-Cancino

Departamento de Microbiología, Universidad de Concepción, Concepción, Región del Biobío, Chile

Tel: +56 41 220-4144 / Fax: +56 41 224-5975

E-mail: apgarcia@udec.cl

amplified in different sources, including food and water, with no success in the isolation of this bacterium through cultivation<sup>2,4,5</sup>. *H. pylori* infects more than 50% of the world population and is considered the main predisposing factor to the development of gastric cancer<sup>6</sup>. In Chile, its prevalence is 73% in children older than 17 years and is significantly higher in individuals of lower socioeconomic classes and males<sup>7</sup>.

*H. pylori* infections are a public health problem worldwide, and it features in the list of pathogens with high priority by WHO<sup>8</sup>, which makes us ask: What makes *H. pylori* such an effective pathogen? One way to answer this question is by saying that this pathogen has multiple virulence factors, which ensure its effective colonization. In addition, it is a facultative intracellular microorganism able to invade and multiply itself inside vacuoles of eukaryotic cells, including yeasts of the genus *Candida*<sup>2,9,10</sup>.

There is evidence that *H. pylori* and *Candida* share habits in areas of the human anatomy, such as in the mouth, which is the anatomical site for the entry of microorganisms that colonize the digestive apparatus, in addition to the fact that both microorganisms can survive in the acidic environment of the stomach<sup>11-13</sup>, which would favor their interaction and the internalization of the bacterium in the yeast, which would grant it protection, nutrition, and a transmission medium that would allow the bacteria to survive outside the human gastrointestinal environment since yeast can survive in different environments, including in conditions with a lack of nutrients<sup>2,10,14-16</sup>.

The high prevalence of gastrointestinal diseases associated with *H. pylori*, the increased resistance of this pathogen to antibiotic treatment<sup>17,18</sup>, and the lack of knowledge regarding its means of transmission make it necessary to obtain evidence that allows developing effective strategies to reduce its spread and, consequently, the prevalence of pathologies associated to its infection. For this reason, the goal of this study was to detect the presence of *H. pylori* in intracellular yeasts of the genus *Candida* of oral origin isolated from young graduates of the University of Concepción, Chile.

## METHODS

This study was approved by the ethics committee of the University of Concepción, under number E-224-18. Prior to the sample collection, each participant received information, oral and written, about the importance of

the study and how the sample would be collected. In addition, we explained they could leave the study at any stage. We carried out a quantitative, observational, transversal study. Our population consisted of undergraduate students of the University of Concepción.

### Inclusion criteria

Signing the informed consent form.

### Exclusion criteria

Treatment with antifungal agents a month before or at the time of sample collection.

### Questionnaire

The people who participated in the study individually filled out a questionnaire with the questions: 1) Have you presented or do you present stomach discomfort in the last 30 days? 2) What kind of discomfort is/was that? (Gastritis/Reflux/Abdominal pain/Nausea/Vomiting/Diarrhea), 3) What was the duration of the discomfort? (Hours/A day/ More than a day/ More than a week), 4) Do you wash your hands before and after using the restroom?, 5) Do you drink potable water, bottled water, or non-potable water?, 6) Were you diagnosed with any gastrointestinal pathology? 7) Have you ever been diagnosed with *H. pylori*?, 8) Has any of your relatives ever been diagnosed *H. pylori*?

### Sample collection

Participants were instructed not to brush their teeth for at least two hours before the sample collection. The sample was collected orally with a cotton swab, which was scraped on the cheeks, soft palate, tongue's surface, underneath the tongue, and gums, according to Matamala-Valdés et al.<sup>2</sup>. Subsequently, each swab was placed in test tubes with 5 mL of liquid YPD (Difco, USA), which were incubated in aerobic conditions for 48 hours. After that, each sample was seeded on Sabouraud Dextrose Agar plates (Difco, USA) supplemented with Chloramphenicol (Oxoid, United Kingdom) rubbing the swab across the entire surface of the culture medium; the plates were incubated at 37°C for 48 hours under aerobic conditions. Subsequently, we confirmed the growth of pure yeasts through wet Gram staining. From the primary culture, two consecutive seedings were carried out in Sabouraud Dextrose Agar (Difco, USA), supplemented with Chloramphenicol (Oxoid, United Kingdom) to ensure the purity of the sample; each re-seeding was followed by wet Gram staining examination.

### Detection of bacterium-like organelles (BLOs) on the inside of fungal cells by optical microscopy.

We carried out a wet examination in yeast colonies randomly chosen in search of BLOs inside the fungal cells, using as a negative control *C. albicans* ATCC 90028. Each colony was placed in 20 µL of saline solution and observed using an optical microscope, with a 100X objective lens.

### Tests for the identification of *Candida* spp.

We took an inoculum from the crops of pure yeast in Sabouraud Agar, which was then sown on CHROMagar *Candida* (Difco, USA) and incubated under aerobic conditions at 37°C for 24-48 hours. The interpretation was performed according to the manufacturer's instructions. Subsequently, the results were confirmed using the identification system API®*Candida*, following the manufacturer's recommendations (BIOMÉRIEUX, France).

### DNA extraction

The total DNA from the yeast isolated from the oral swab samples and controls was extracted from pure cultures obtained in Sabouraud Agar, using the commercial kit UltraClean® Microbial DNA Isolation (MO BIO lab) and following the manufacturer's instructions. The quantification and degree of purity of the DNA extracted was obtained by spectrophotometry (TECAN, Switzerland)

### Genotyping of *Helicobacter pylori*

The amplification of the 16S rDNA of the *H. pylori* and the electrophoresis of the amplified products was carried out according to the protocol described by Matamala-Valdés et al.<sup>2</sup>.

### Statistical analysis

The data obtained in this study were processed using the XLSTAT software 2017. We used Fisher's exact test to determine the relationship between the categorical variables. The level of significance used was 0.05, considering a value significant whenever  $p < 0.05$ .

## RESULTS

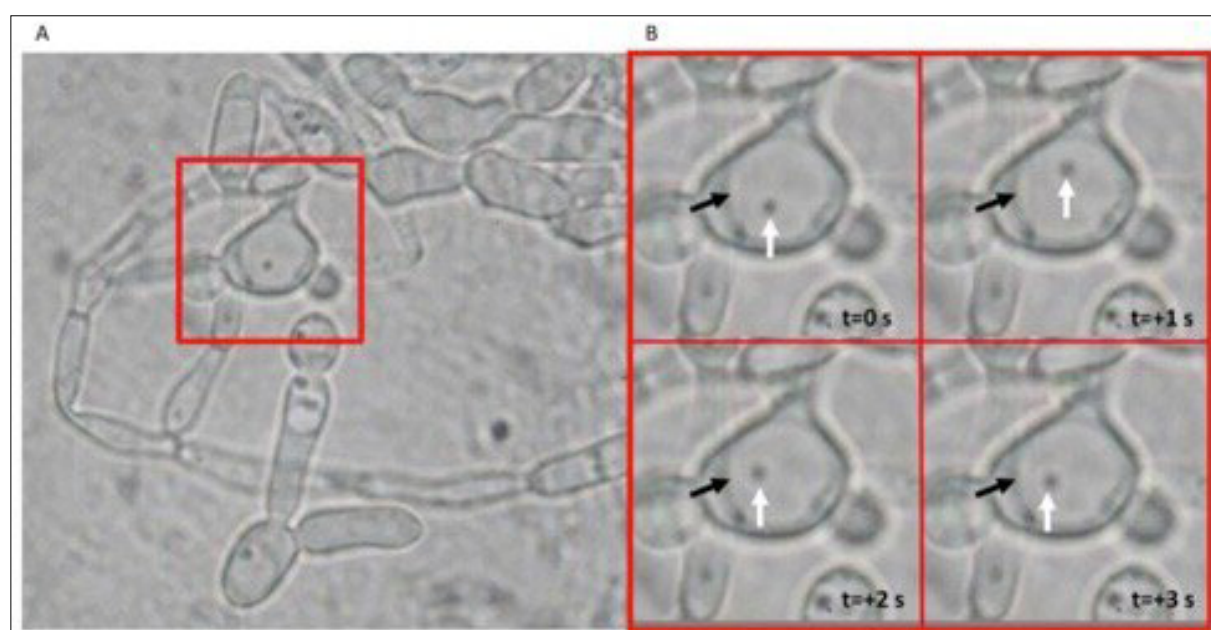
### Prevalence of oral yeasts

A total of 72 students participated in the study, 30 of whom were women and 42 men. Of the 72 oral swab samples, there was a prevalence of 33.3% (24/72) of oral yeasts. The yeast species isolated with greater frequency in the oral mucosa was *C. albicans* with 79.2%, followed by *C. dubliniensis* with 12.4%, *C. krusei* with 4.2%, and *C. tropicalis* with 4.2%.

### Observation by optical microscopy

The observation of the samples by optical microscopy revealed the presence of BLOs in 15/24 (63%) of the isolated yeast samples; the BLOs were observed quickly and continuously detaching themselves inside the yeast vacuoles (Figure 1).

FIGURE 1



Kimberly Sánchez-Alonzo

## Amplification of the 16S rDNA gene

We amplified the 16S rDNA gene of the *H. pylori* in 63% of the oral yeasts (15/24), which was in line with the 15 samples of yeast in which the BLOs were observed (Figure 2).

## Results of the applied research

Regarding the results obtained from the questionnaire with the 72 participants, we found that 80% of the 15 participants from whom oral yeasts with *H. pylori* were isolated had had some stomach discomfort in the thirty days prior to the sample collection, and the most frequent type of discomfort was abdominal pain and gastritis, with 33% and 27%, respectively (Table 1). In addition, out of the 15 participants with oral yeasts positive for *H. pylori*, 20% had been diagnosed at some time with *H. pylori*, and 33.3% had a relative with a diagnosis of *H. pylori* infection at some time in their lives (Tables 2 and 3).

## Association of intracellular *H. pylori* in yeasts and information obtained from the research.

Fisher's exact test showed that there was no statistically significant association between the presence of stomach discomfort and the amplification of the 16S rDNA gene of the *H. pylori* in the DNA extracted from the oral yeasts ( $p=0.569$ ). No statistically significant association was observed between the positive diagnosis for *H. pylori*, and the amplification of the 16S rDNA gene for the *H. pylori*, also based on the total DNA from oral yeasts ( $p=0.154$ ). Finally, when applying

Fisher's exact test for the association of the diagnosis of a patient's family member with *H. pylori* and the amplification of the 16S rDNA gene for *H. pylori*, we obtained a p-value of 1.0, indicating that there was no statistical significance for this association.

## DISCUSSION

The coexistence of *H. pylori* and *Candida* spp. has existed for a long time; however, the benefits the bacteria receive when entering the yeast remain unknown<sup>2,10,14,19</sup>. Furthermore, cases have been reported of bacteria that enter fungi to protect themselves from environmental stress. An example of that is the endosymbiotic relationship between

**TABLE 1.** PERCENTAGE OF STOMACH DISCOMFORT REPORTED BY STUDY PARTICIPANTS, FROM WHOM ORAL YEASTS WITH INTRACELLULAR *H. PYLORI* WERE ISOLATED.

| Type of discomfort        | Frequency | Percentage |
|---------------------------|-----------|------------|
| Abdominal pain            | 5         | 33%        |
| Gastritis                 | 4         | 27%        |
| Reflux                    | 1         | 7%         |
| Vomiting                  | 0         | 0%         |
| Nausea                    | 1         | 7%         |
| Diarrhea                  | 1         | 7%         |
| Did not report discomfort | 3         | 19 %       |
| Total                     | 15        | 100%       |

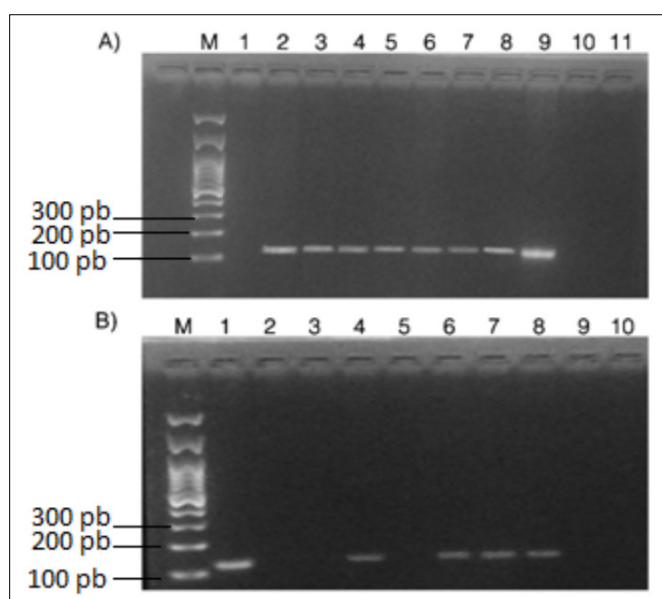
**TABLE 2.** RELATIONSHIP BETWEEN PARTICIPANTS DIAGNOSED WITH INFECTION BY *H. PYLORI* AND THE MOLECULAR IDENTIFICATION OF THIS PATHOGEN IN YEASTS FROM THE ORAL CAVITY.

| Diagnosis | Samples with amplified 16S rDNA of the <i>H. Pylori</i> | Samples without amplified 16S rDNA of the <i>H. Pylori</i> | Total |
|-----------|---|--|-------|
| Yes       | 1   | 2  | 3     |
| No        | 10  | 2  | 12    |
| Total     | 11  | 4  | 15    |

**TABLE 3.** RELATIONSHIP BETWEEN PARTICIPANTS' FAMILY MEMBERS DIAGNOSED WITH AN INFECTION BY *H. PYLORI* AND THE MOLECULAR IDENTIFICATION OF THIS PATHOGEN IN YEASTS FROM THE ORAL CAVITY.

| Diagnosis | Samples with amplified 16S rDNA of the <i>H. pylori</i> | Samples without amplified 16S rDNA of the <i>H. pylori</i> | Total |
|-----------|---|--|-------|
| Yes       | 4   | 1  | 5     |
| No        | 7   | 3  | 10    |
| Total     | 11  | 4  | 15    |

**FIGURE 2**



Kimberly Sánchez-Alonzo



the *Candidatus glomeribacter* and the *Micorriza arbuscular*<sup>20,21</sup>.

The yeasts of the *Candida* genus are part of the human microbiota and are distributed in greater volume in the oral cavity on the tongue's surface, the palate and in the oral mucosa<sup>22,23</sup>; on the other hand, the presence of *H. pylori* in the oral cavity was detected by molecular tests<sup>2,10,14,19,24</sup>. However, there are scarce cases of these bacteria being isolated on this anatomical site, assuming that DNA fragments of *H. pylori* can become lodged in the mouth through the reflux in patients infected at the gastric level<sup>25</sup> or that it is temporarily lodged in the mouth, indicating that the oral microenvironment does not provide the conditions necessary for the survival of the bacteria, which would force *H. pylori* to create strategies to protect itself from unfavorable environments, such as entering oral yeasts, a phenomenon demonstrated by research conducted by Siavoshi *et al.*<sup>10,14</sup> and Matamala-Valdés *et al.*<sup>2</sup>.

In the general population, the percentage of people with oral *Candida* spp. varies between 17-75%<sup>17,24</sup>, and 75% of the isolates from the oral mucosa correspond to *C. albicans*, followed by *C. tropicalis* (8%), and *C. krusei* (3-6%)<sup>23</sup>. In the present study, the isolated species in order of frequency were *C. albicans* with 79.2%, followed by *C. dubliniensis* with 12.5%, *C. krusei* (4.2%), and *C. tropicalis* with 4.2%, in agreement with the data presented by the bibliography reviewed.

Regarding the observation by optical microscopy of the wet examination of the oral yeasts with *H. pylori*, we found that the bacteria movement was limited to the size of the vacuole; in addition, the presence of intravacuolar *H. pylori* was observed both in yeast

obtained from primary culture and on re-seedings, which suggests that this microorganism is not only capable of entering the yeast, but also possibly of reproducing inside the fungal cell. This same essay allowed us to observe that there was an increase in the size of the yeast vacuoles with *H. pylori* when compared to those without the presence of the bacteria; (Figure 1).

### Acknowledgments

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### Ethical aspects

The authors of this study declare there are no conflicts of interest. This research was approved by the Scientific Ethics Committee of the Faculty of Biological Sciences of the University of Concepción, Concepcion - Chile, under number E-224-18 and was approved on 7 March 2018.

### Author's Contribution

Collection: Kimberly Sánchez-Alonzo, Cristian Parra-Sepúlveda, Lorena Vergara. Processing of samples: Kimberly Sánchez-Alonzo, Cristian Parra-Sepúlveda, Humberto Bernasconi. Analysis of results and writing of the scientific paper: Kimberly Sánchez-Alonzo, Cristian Parra-Sepúlveda, Apolinaria García-Cancino. Management for funding: Lorena Vergara, Apolinaria García-Cancino.

## RESUMO

**INTRODUÇÃO:** Quase 73% da população chilena estão infectadas pelo *Helicobacter pylori* (*H. pylori*), fator predisponente ao câncer gástrico. Estudos recentes demonstraram a presença desse patógeno em leveduras, sugerindo que esse fato pode influenciar diretamente a falha de um tratamento, transmissão e reinfeção.

**OBJETIVO:** Detectar a presença de *H. pylori* em leveduras orais isoladas de estudantes da Universidade de Concepción (Chile).

**MÉTODOS:** 72 amostras, obtidas da cavidade oral utilizando cotonetes, foram incubadas em caldo YPD por 48h a 37°C e posteriormente sementes em ágar Sabouraud Dextrose mais cloranfenicol na mesma temperatura e ao mesmo tempo. Leveduras isoladas foram observadas microscopicamente (montagem úmida e corada por Gram) e identificadas utilizando técnicas microbiológicas. A detecção intracelular de *H. pylori* foi realizada pela amplificação do 16S rDNA por PCR.

**RESULTADOS:** Leveduras orais foram detectadas em 24 amostras (33,3%), sendo *C. albicans* (79,2%), a espécie mais frequente seguida por *C. dubliniensis* (12,4%), *C. krusei* (4,2%) e *C. tropicalis* (4,2 %). Quando analisadas por PCR, 15 das 24 leveduras orais 62,5% foram positivas para o *H. pylori* 16S rDNA. Dos 15 indivíduos positivos para leveduras que abrigam *H. pylori*, 81% deles relataram desconforto estomacal e a presença da bactéria foi diagnosticada em algum momento em 20% deles.

**CONCLUSÃO:** A presença intracelular do *H. pylori* em leveduras orais sugere uma relação endossimbiótica desses microrganismos, o que poderia favorecer a transmissão e a reinfeção do *H. pylori* no trato gastrointestinal.

**PALAVRAS-CHAVE:** *Helicobacter pylori*. *Candida*. Boca.






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# Evaluation of peptidylarginine deiminase 4 and PADI4 polymorphisms in sepsis-induced acute kidney injury

 Nara Aline Costa<sup>1</sup>  
 Bertha Furlan Polegato<sup>2</sup>  
 Amanda Gomes Pereira<sup>3</sup>  
 Sérgio Alberto Rupp de Paiva<sup>4</sup>  
 Ana Lúcia Gut<sup>2</sup>  
 André Luís Balbi<sup>2</sup>  
 Daniela Ponce<sup>5</sup>  
 Leonardo Antonio Mamede Zornoff<sup>4</sup>  
 Paula Schmidt Azevedo<sup>2</sup>  
 Marcos Ferreira Minicucci<sup>2</sup>

1. Professora Adjunta da Faculdade de Nutrição da Universidade Federal de Goiás – UFG, Goiânia, GO, Brasil

2. Professor(a) Associado(a) da Faculdade de Medicina de Botucatu – Unesp, Botucatu, SP, Brasil

3. Aluna do Programa de Pós-graduação em Fisiopatologia em Clínica Médica – Unesp, São Paulo, SP, Brasil

4. Professor Titular da Faculdade de Medicina de Botucatu – Unesp, Botucatu, SP, Brasil

5. Médica Livre-docente e Coordenadora do Programa de Pós-graduação em Fisiopatologia em Clínica Médica – Unesp, São Paulo, SP, Brasil

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## SUMMARY

**BACKGROUND:** The aim of this study is to evaluate the peptidylarginine deiminase 4 (PAD 4) concentration and PADI4 polymorphisms as predictors of acute kidney injury (AKI) development, the need for renal replacement therapy (RRT), and mortality in patients with septic shock.

**METHODS:** We included all individuals aged  $\geq 18$  years, with a diagnosis of septic shock at ICU admission. Blood samples were taken within the first 24 hours of the patient's admission to determine serum PAD4 concentration and its PADI4 polymorphism (rs11203367 and rs874881). Patients were monitored during their ICU stay and the development of SAKI was evaluated. Among the patients in whom SAKI developed, mortality and the need for RRT were also evaluated.

**RESULTS:** There were 99 patients, 51.5% of whom developed SAKI and of these, 21.5% needed RRT and 80% died in the ICU. There was no difference between PAD4 concentration ( $p = 0.116$ ) and its polymorphisms rs11203367 ( $p = 0.910$ ) and rs874881 ( $p = 0.769$ ) in patients in whom SAKI did or did not develop. However, PAD4 had a positive correlation with plasma urea concentration ( $r = 0.269$  and  $p = 0.007$ ) and creatinine ( $r = 0.284$  and  $p = 0.004$ ). The PAD4 concentration and PADI4 polymorphisms were also not associated with RRT and with mortality in patients with SAKI.

**CONCLUSION:** PAD4 concentration and its polymorphisms were not associated with SAKI development, the need for RRT, or mortality in patients with septic shock. However, PAD4 concentrations were associated with creatinine and urea levels in these patients.

**KEYWORDS:** Acute kidney injury. Protein-arginine deiminase type 4. Sepsis. Mortality. Dialysis.

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CORRESPONDING AUTHOR: Nara Aline Costa

Faculdade de Nutrição, Universidade Federal de Goiás (UFG)

Rua 227, quadra 68 s/n, Setor Leste Universitário, Goiânia – GO, CEP: 74605-080 – Tel.: 55 62 3811-6000

E-mail: nara costa@ufg.br

## INTRODUCTION

Sepsis-induced acute kidney injury (SAKI) represents one of the most common complications of sepsis and is associated with a worse prognosis and with mortality rates ranging from 50% to 70%<sup>1</sup>. Despite intense research, there is currently no effective therapy or preventive measures for SAKI<sup>1</sup>. The physiopathology of SAKI is multifactorial and complex, involving changes in renal hemodynamics, endothelial dysfunction, infiltration of inflammatory cells into the renal parenchyma, intraglomerular thrombosis, and tubular cell necrosis<sup>2</sup>. Indeed, renal inflammation and injury are mediated by the upregulation of proinflammatory cytokines followed by leukocyte infiltration, including T lymphocytes, natural killer cells, and neutrophils<sup>2</sup>. It should be reinforced that infiltrating neutrophils play a major role in renal tubular inflammation and cell death.<sup>2</sup> In fact, blocking or neutralizing neutrophils has been shown to attenuate the severity and duration of ischemic acute kidney injury (AKI)<sup>3,4</sup>.

Thus, the understanding of pathways that modulate the inflammatory process could improve the current knowledge of SAKI physiopathology and identify new therapeutic targets. In this scenario, the citrullination process deserves to be highlighted because it has been identified in several inflammatory diseases<sup>5</sup>. Citrullination is the posttranslational modification of peptidyl arginine to peptidyl citrulline, catalyzed by the family of enzymes peptidyl arginine deiminase (PAD)<sup>6</sup>. In humans and rodents, there are five PAD subtypes. Among them, PAD4 has been widely studied in innate immunity and autoimmune diseases as multiple sclerosis, lupus, ulcerative colitis, and rheumatoid arthritis<sup>7</sup>.

PAD4 was first identified in the nucleus of a neutrophil-like cell line (HL-60)<sup>7</sup>, and PAD4 mediated citrullination of core histones (H3, H4, and H2A) is critical for neutrophil extracellular traps (NETs) formation after bacterial infection<sup>8</sup>. In the kidney, PAD2 and PAD4 messenger RNAs are present, although only PAD4 protein expression has been detected<sup>9,10</sup>. Ham *et al.*<sup>11</sup>, demonstrated that PAD4 plays an important role in a model of renal ischemia-reperfusion injury by increasing tubular inflammatory response. Based on previous data published by our group, using the same dataset, higher PAD4 concentrations were associated with lower ICU survival in patients with septic shock<sup>12</sup>.

Despite PAD4 concentration, *PADI4* polymorphisms could influence enzyme activity. In this context, some polymorphisms of the *PADI4* gene have been found

in humans. The *PADI4* gene is located on the short arm of chromosome 1 at position 36.13, and functional polymorphisms, such as rs11203367 and rs874881, have been associated with susceptibility to rheumatoid arthritis<sup>13,14</sup>.

However, to our knowledge, no clinical studies have evaluated the influence of PAD4 concentration and its polymorphisms in SAKI development. Thus, the aim of the current study was to evaluate PAD4 concentration and *PADI4* polymorphisms as predictors of SAKI development, and among these patients, the need for renal replacement therapy (RRT) and mortality.

## METHODS

### Study population

This prospective observational clinical study was a subanalysis of a previous study by our group that analyzed oxidative stress markers as early predictors of septic shock mortality and SAKI development<sup>15</sup>. We included patients admitted to the Intensive Care Unit (ICU) of our institution with the diagnosis of septic shock, from May 2014 to June 2015.

We included in the study all individuals with the diagnosis of septic shock at ICU admission, over 18 years of age, of both sexes and requiring vasopressor support for at least 24 hours. The diagnosis of septic shock and acute kidney injury was made by the medical team according to standard guidelines<sup>16,17</sup>. Baseline creatinine was characterized as the lowest creatinine value in the past 6 months before AKI or, the lowest value achieved during hospitalization in the absence of dialysis<sup>18,19</sup>. CKD was defined according to CKD Epidemiology Collaboration equation (CKD-EPI)<sup>20</sup>.

The exclusion criteria were delayed diagnosis of septic shock (time greater than 24 h), the presence of other types of shock, noradrenaline dose > 2.0 µg/kg/min, inability to provide consent, pregnancy, confirmed brain death, and the need for palliative care. Moreover, we did not include patients with AKI at ICU admission, stage 4 or 5 CKD (creatinine clearance less than 30 mL/min/1.73 m<sup>2</sup>).

Demographic, clinical and laboratory data were collected upon ICU admission. Blood samples were taken within the first 24 hours of the patient's admission to determine serum PAD4 concentration and its polymorphism *PADI4* (rs11203367) and (rs874881). The development of SAKI was evaluated and among the patients in whom SAKI developed, mortality and need for RRT were also evaluated.

## Laboratory Analysis

Laboratory tests such as CRP, albumin, lactate, urea and creatinine were measured with the dry chemistry method. The hemograms were performed with a Coulter STKS hematological auto analyzer.

### Serum PAD4 concentration

For the determination of serum concentration of PAD4, we used enzyme-linked immunosorbent assay (Cloud-Clone Corp, Houston, TX, USA). All instructions on the manufacturer's kit were followed, and the sensitivity value adopted was 0.137 ng/mL.

### PADI4 gene polymorphism

From whole blood samples, the DNA was isolated, its integrity was confirmed on 1% agarose gel, and the concentration was obtained by Nanodrop 8000 spectrophotometer (Thermo Scientific, Waltham, MA, USA). In addition, the TaqMan Open Array (Applied Biosystem, Foster City, CA, USA) was used according to the manufacturer's instructions for the genotyping of the polymorphisms of the *PAD4* gene rs11203367 and rs87481.<sup>21</sup>

## Statistical Analysis

Data are expressed as mean  $\pm$  standard deviation, median (including the lower and upper quartiles), or percentage. Statistical comparisons between two groups for continuous variables were performed using the *t*-test for parameters with a normal distribution. If data were not normally distributed, comparisons between two groups were performed using the Mann-Whitney *U* test. The Fisher test or the chi-square test was used for all categorical data. The Spearman correlation was performed to analyze the association between continuous variables. The Hardy-Weinberg equilibrium was determined using the chi-square test. Data analysis was performed using SigmaPlot software for Windows v12.0 (Systat Software Inc., San Jose, CA, USA). Values of *P* < 0.05 were considered statistically significant.

## RESULTS

During the study, 150 consecutive patients were admitted to the ICU with the diagnosis of septic shock. However, 27 patients were excluded due to the presence of AKI at ICU admission; 12 because of a delay in septic shock diagnosis; 4 because of the presence of advanced chronic kidney disease; 3 because PAD4

**TABLE 1.** DEMOGRAPHIC, LABORATORY, AND PADI4 POLYMORPHISM DATA OF 99 PATIENTS WITH SEPTIC SHOCK

| Variables                 | SAKI development   |                    | P      |
|---------------------------|--------------------|--------------------|--------|
|                           | Yes (n = 51)       | No (n = 48)        |        |
| Age (years)               | 67 (59 -75)        | 64 (51-71)         | 0.093  |
| Male, n (%)               | 27 (53)            | 28 (58)            | 0.736  |
| APACHE II score           | 18.8 $\pm$ 6.0     | 16.5 $\pm$ 6.8     | 0.070  |
| SOFA score                | 10.4 $\pm$ 2.7     | 8.8 $\pm$ 2.3      | 0.002  |
| Sepsis focus, n (%)       |                    |                    | 0.203  |
| Respiratory               | 35 (69)            | 23 (48)            |        |
| Abdominal                 | 11 (22)            | 12 (25)            |        |
| Urinary                   | 1 (2)              | 3 (6)              |        |
| Outros                    | 4 (7)              | 10 (21)            |        |
| Mortality ICU, n (%)      | 41 (80)            | 28 (58)            | 0.003  |
| PADI4 (rs11203367), n (%) |                    |                    | 0.910  |
| CT                        | 26 (51)            | 23 (48)            |        |
| CC                        | 17 (33)            | 18 (37.5)          |        |
| TT                        | 8 (16)             | 7 (14.5)           |        |
| PADI4 (rs874881), n (%)   |                    |                    | 0.769  |
| CG                        | 28 (55)            | 23 (48)            |        |
| GG                        | 8 (16)             | 8 (17)             |        |
| CC                        | 15 (29)            | 17 (35)            |        |
| PAD4 (ng/mL)              | 4.6 (3.1- 6.7)     | 3.8 (1.9 - 6.2)    | 0.116  |
| Lactate (mmol/L)          | 2.3 (1.4 - 3.5)    | 2.3 (1.2 - 3.8)    | 0.890  |
| Hemoglobin (g/dL)         | 10.7 $\pm$ 2.0     | 11.4 $\pm$ 2.1     | 0.072  |
| Hematocrit (%)            | 32.2 $\pm$ 6.1     | 34.0 $\pm$ 6.1     | 0.155  |
| Leucocytes (103/mm3)      | 17.0 (13.2 - 22.4) | 16.5 (11.0 - 24.6) | 0.872  |
| CRP (mg/dL)               | 36.5 (28.0 - 44.1) | 30.5 (8.2 - 35.5)  | 0.030  |
| Albumin (g/dL)            | 2.3 (2.0 - 2.5)    | 2.1 (1.8 - 2.6)    | 0.427  |
| Urea (mg/dL)              | 101 (68 - 160)     | 53 (33 - 85)       | <0.001 |
| Creatinine (mg/dL)        | 2.0 (1.6 - 2.6)    | 0.8 (0.5 - 1.1)    | <0.001 |

APACHE II = Acute Physiology and Chronic Health Evaluation, SOFA = Sequential Organ Failure Assessment, ICU = intensive care unit, PADI4 = polymorphisms peptidylarginine deiminase 4, PAD4 = peptidylarginine deiminase 4, CRP = C-reactive protein. Data are expressed as the mean  $\pm$  SD, median (including the lower and upper quartiles) or percentage.

concentration was below assay sensitivity; and 5 had technical problems with polymorphism analysis. Thus, we evaluated 99 patients.

The mean age was 63.6  $\pm$  14.4 years, 56% were male, and the median length of ICU stay was 9 (4 to 17) days. The mortality rate during the ICU stay was 69.9%. Median PAD4 serum concentration was 4.4 (2.5 to 6.2) ng/mL. Among these patients with septic shock, SAKI developed in 51.5% during the ICU stay; of these, 21.5% required RRT and 80% died.

The genotype frequencies for the rs11203367 polymorphism were 49.59% for CT, 35.4% for CC, and 15.1% for TT; for the rs874881 polymorphism, they were 51.5% for CG, 16.2% for GG, and 32.3% for CC. These

**TABLE 2.** DEMOGRAPHIC, PAD 4 SERUM CONCENTRATION, AND PADI4 POLYMORPHISM DATA OF 51 PATIENTS WITH SAKI

| Variables                 | Mortality ICU |               | P     |
|---------------------------|---------------|---------------|-------|
|                           | Yes (n = 41)  | No (n = 10)   |       |
| Age (years)               | 66.5 + 12.8   | 64.5 + 11.4   | 0.624 |
| Male, n (%)               | 21 (51)       | 6 (60)        | 0.731 |
| APACHE II score           | 19.7 + 6.1    | 15.4 + 4.3    | 0.043 |
| SOFA score                | 10.8 + 2.7    | 8.6 + 1.7     | 0.020 |
| Sepsis focus, n (%)       |               |               | 0.507 |
| Respiratory               | 29 (71)       | 6 (60)        |       |
| Abdominal                 | 7 (17)        | 4 (40)        |       |
| Urinary                   | 1 (2)         | 0 (0)         |       |
| Others                    | 4 (10)        | (0)           |       |
| PAD 4 (ng/mL)             | 4.7 (3.1-6.8) | 3.9 (2.4-7.2) | 0.678 |
| PADI4 (rs11203367), n (%) |               |               | 0.780 |
| CT                        | 20 (49)       | 6 (60)        |       |
| CC                        | 14 (34)       | 3 (30)        |       |
| TT                        | 7 (17)        | 1 (10)        |       |
| PADI4 (rs874881), n (%)   |               |               | 0.563 |
| CG                        | 21 (51)       | 7 (70)        |       |
| GG                        | 7 (17)        | 1 (10)        |       |
| CC                        | 13 (32)       | 2 (20)        |       |

APACHE II = Acute Physiology and Chronic Health Evaluation, PAD4 = peptidylarginine deiminase 4, PADI4 = polymorphisms peptidylarginine deiminase 4, SOFA = Sequential Organ Failure Assessment. Data are expressed as the mean ± standard deviation, median (including the lower and upper quartiles) or percentage.

**TABLE 3.** DEMOGRAPHIC, PAD4 SERUM CONCENTRATION, AND PADI4 POLYMORPHISM DATA OF 51 PATIENTS WITH SAKI

| Variables                 | RRT ICU            |                  | P     |
|---------------------------|--------------------|------------------|-------|
|                           | Yes (n = 11)       | No (n = 40)      |       |
| Age (years)               | 63.2 + 14.1        | 67.1 + 12.1      | 0.365 |
| Male, n (%)               | 4 (36)             | 23 (58)          | 0.367 |
| APACHE II score           | 21.8 + 6.4         | 18.0 + 5.7       | 0.061 |
| SOFA score                | 12.0 (12.0 - 15.0) | 9.5 (8.0 - 11.0) | 0.003 |
| PAD4 (ng/mL)              | 4.6 (3.1 - 9.3)    | 4.5 (2.8 - 6.1)  | 0.384 |
| PADI4 (rs11203367), n (%) |                    |                  | 0.102 |
| CT                        | 4 (36.5)           | 22 (55)          |       |
| CC                        | 4 (36.5)           | 14 (35)          |       |
| TT                        | 3 (27)             | 4 (10)           |       |
| PADI4 (rs874881), n (%)   |                    |                  | 0.094 |
| CG                        | 4 (36.5)           | 24 (60)          |       |
| GG                        | 4 (36.5)           | 4 (10)           |       |
| CC                        | 3 (27)             | 12 (30)          |       |

APACHE II = Acute Physiology and Chronic Health Evaluation; ICU = intensive care unit, SOFA = Sequential Organ Failure Assessment, PAD 4 = peptidylarginine deiminase 4; PADI4: polymorphisms peptidylarginine deiminase 4, RRT, renal replacement therapy. Data are expressed as the mean ± standard deviation, median (including the lower and upper quartiles) or percentage.

frequencies are consistent with those expected under the Hardy-Weinberg equilibrium.

The demographic and laboratory data are presented in Table 1.

Patients with SAKI had higher Sequential Organ Failure Assessment (SOFA) score, a higher serum concentration of C-reactive protein (CRP), urea, and creatinine, and a higher mortality rate. There were no differences between PAD4 concentrations and its polymorphisms rs11203367 and rs87481 in patients in whom SAKI developed or not. Nevertheless, it is relevant to note that the serum concentration of PAD4 had a positive correlation with plasma urea ( $r = 0.269$ ;  $p = 0.007$ ) and creatinine concentration ( $r = 0.284$ ;  $p = 0.004$ ).

Patients with SAKI who died in the ICU had higher Acute Physiology and Chronic Health Evaluation (APACHE II) and SOFA scores. However, there were no differences between PAD4 concentrations and its polymorphisms rs11203367 and rs874881 in patients who died during the ICU stay (Table 2).

Patients with SAKI who needed RRT had a higher SOFA score, but the same association was not observed with PAD4 concentration and PADI4 polymorphisms (Table 3).

## DISCUSSION

The aim of the current study was to evaluate PAD4 concentration and PADI4 polymorphisms as predictors of SAKI development, and among these patients, the need for RRT and mortality. This study showed that PAD4 concentration and its polymorphisms were not associated with SAKI development or with the other outcomes. However, PAD4 concentrations were associated with creatinine and urea levels in patients with septic shock.

SAKI is a frequent complication in patients with sepsis and is associated with adverse outcomes including increased length of hospital stay, development of CKD, and increased risk of death<sup>22,23</sup>. Despite efforts to obtain early diagnosis and treatment of septic shock, mortality rates remain high<sup>15</sup>. In our study, 69.9% of patients with septic shock died. In addition, in patients in whom SAKI developed, the mortality was even greater, representing 80% of the cases. Although very high, our rates are in agreement with data found for Latin America<sup>15,24</sup>.

The pathophysiology of AKI induced by sepsis is multifactorial and complex, and the development of

early and effective therapeutic targets is fundamental. In this scenario, PAD4 could play an important role. Currently, it is believed that the process of renal ischemia-reperfusion contributes to the increase of PAD4 expression. Consequently, there would be a stimulus for the release of proinflammatory cytokines, renal neutrophils infiltration, and histone citrullination, which together would corroborate the formation of NETs. The disconnected formation of NETs seems to stimulate the formation of thrombi, contributing to renal tubular cell necrosis. In addition, patients with septic shock have a high proinflammatory response, reduced immune capacity, and tissue hypoperfusion, which together with increased NET formation create a vicious and extremely lethal cycle<sup>8</sup>. In this context, experimental studies suggest that blocking the action of PAD4 using drugs or genetically has a protective effect against ischemic AKI<sup>25</sup>.

In general, studies available in the literature only address ischemic AKI in experimental models, and this is the first clinical study to evaluate the influence of PAD4 and its polymorphisms in SAKI. In this study, AKI developed in half of the patients during hospitalization, which contributes to septic shock severity and survival reduction. Despite this, we did not observe a difference in the concentration of PAD4 between the individuals in whom AKI developed and those who died. This response is different from what we found in our previous study, with the same dataset, in which PAD4 concentration was associated with increased mortality in patients with septic shock<sup>12</sup>. This different performance of PAD4 as a biomarker suggests that PAD4 concentration is more important in sepsis than in AKI pathophysiology. Importantly, despite the lack of significant results, we did not rule out the influence of PAD4 in the development and worse evolution of AKI because its higher concentration was associated with increased levels of creatinine and urea in patients with septic shock.

It is already known that functional polymorphisms could influence enzyme activity and patient evolution. In this work, we evaluated only two *PADI4* polymorphisms (rs 11203367, rs 87481) and none of them had an association with the development of SAKI, or the need for RRT and mortality. In addition, our previous study also showed that other *PADI4* polymorphisms (rs 11203366, rs 2240340, rs 1748033) were not associated with mortality in patients with septic shock<sup>12</sup>. We also could not exclude the participation of other *PADI4* polymorphisms in SAKI development and outcomes.

Some limitations of this study should be considered. We only included patients from a single center and our sample size was relatively small. Regarding the diagnosis of AKI, it was based on serum creatinine values and not on urine output. Moreover, the only inflammation marker that we measured was CRP and we did not evaluate the formation of NETs. Despite these limitations, we strongly believe that our data contribute to a better knowledge of inflammatory pathways in patients with SAKI.

## CONCLUSION

In conclusion, PAD4 concentrations were associated with creatinine and urea levels in patients with septic shock. However, PAD4 concentration and its polymorphisms were not associated with SAKI development, the need for RRT, and mortality in these patients.

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## Author's Contribution

Nara Aline Costa: concept, data collection, and drafting of the original article

Bertha Furlan Polegato: data collection, formal analysis, and review of the original article

Amanda Gomes Pereira: data collection and methodology

Sergio Alberto Rupp de Paiva: formal analysis, investigation, methodology, and review of the original article

Ana Lúcia Gut: formal analysis and methodology

André Luís Balbi: formal analysis and methodology

Daniela Ponce: formal analysis, investigation, methodology, and review of the original article

Leonardo Antônio Mamede Zornoff: formal analysis, drafting, and review of the original article

Paula Schmidt Azevedo: formal analysis and methodology

Marcos Ferreira Minicucci: formal analysis, project managing, supervision, drafting, and review of the original article



## RESUMO

**OBJETIVO:** Avaliar a concentração da peptidilarginina deiminase 4 (PAD4) e os polimorfismos de PADI4, como preditores de desenvolvimento de lesão renal aguda, necessidade de terapia renal substitutiva (TRS) e mortalidade em pacientes com choque séptico.

**MÉTODOS:** Foram incluídos indivíduos com idade  $\geq 18$  anos, com diagnóstico de choque séptico na admissão na Unidade de Terapia Intensiva (UTI). Amostras de sangue foram coletadas nas primeiras 24 horas após a admissão do paciente para determinar a concentração sérica de PAD4 e seus polimorfismos PADI4 (rs11203367 e rs874881). Os pacientes foram acompanhados durante a internação na UTI e tiveram avaliados desenvolvimento da lesão renal aguda séptica (Sepsis-induced acute kidney injury - Saki), necessidade TRS e mortalidade.

**RESULTADOS:** Foram avaliados 99 pacientes; 51,5% desenvolveram Saki e, desses, 21,5% necessitaram de TRS e 80% morreram na UTI. Não houve diferença entre a concentração de PAD4 ( $p=0,116$ ) e seus polimorfismos rs11203367 ( $p=0,910$ ) e rs874881 ( $p=0,769$ ) entre os pacientes. No entanto, o PAD4 apresentou correlação positiva com a concentração plasmática de ureia ( $r=0,269$ ;  $p=0,007$ ) e creatinina ( $r=0,284$ ;  $p=0,004$ ). A concentração de PAD4 e os polimorfismos da PADI4 também não foram associados à TRS e à mortalidade em pacientes com Saki.

**CONCLUSÕES:** A concentração de PAD4 e seus polimorfismos não foram associados ao desenvolvimento de Saki, à necessidade de TRS ou à mortalidade em pacientes com choque séptico. No entanto, as concentrações de PAD4 foram associadas às concentrações de creatinina e ureia nesses pacientes.

**PALAVRAS-CHAVE:** Lesão renal aguda. Proteína-arginina desiminase do tipo 4. Seps. Mortalidade. Diálise.

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# EUS-guided gastroenterostomy: Initial experience in a brazilian tertiary center

 Joel Fernandez de Oliveira<sup>1</sup>  
 Martin Andres Coronel Cordero<sup>1</sup>  
 Gustavo Rosa de Almeida Lima<sup>1</sup>  
 Gustavo Andrade de Paulo<sup>1</sup>  
 Marcelo Simas de Lima<sup>1</sup>  
 Bruno da Costa Martins<sup>1</sup>  
 Ulysses Ribeiro Jr.<sup>1</sup>  
 Fauze Maluf-Filho<sup>1</sup>

1. Instituto do Câncer do Estado de São Paulo (Icesp) da Faculdade de Medicina da Universidade de São Paulo (FMUSP), Serviço de Endoscopia Gastrointestinal, São Paulo, SP, Brasil.

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## SUMMARY

**INTRODUCTION:** EUS-guided gastroenterostomy (EUS-GE) is a novel procedure for palliation of malignant gastric outlet obstruction (GOO). Our aim was to evaluate the outcomes of this technique in our initial experience.

**METHODS:** Patients with GOO from our institute were included. Technical success was defined as the successful creation of a gastroenterostomy. Clinical success was defined as the ability to tolerate a soft diet after the procedure. We assessed adverse events and diet tolerance 1 month after the procedure.

**RESULTS:** Three patients were included. Technical and clinical success was achieved in all cases. There were no adverse events and good diet tolerance was observed 1 month after the procedure in the included patients.

**CONCLUSION:** EUS-GE is a promising treatment for patients with GOO.

**KEYWORDS:** Endosonography/methods. Gastric Outlet Obstruction. Gastroenterostomy/methods. Ultrasonography, Interventional/methods.

## INTRODUCTION

Gastric outlet obstruction (GOO) is a clinical condition characterized by nausea, vomiting, postprandial fullness, and abdominal pain as a consequence of mechanical obstruction of the gastrointestinal tract. This is more evident as a potential complication of pancreatic or distal gastric malignancy and can be present in up to 15% to 20% of patients with pancreatic adenocarcinoma<sup>1,2</sup>.

Endoscopic ultrasound-guided gastrojejunostomy or gastroenterostomy (EUS-GE) has recently emerged as a

procedure to treat patients with gastric outlet obstruction as an alternative to surgery or to standard endoscopic enteral stent placement<sup>3</sup>. This procedure is made by inserting a lumen-apposing metal stent (LAMS), from the gastric lumen to the small bowel distal to the obstruction, under endoscopic ultrasound (EUS) guidance.

In 2002, Fritscher-Ravens et al.<sup>4</sup> first introduced the concept of endoscopic gastroenterostomy. However, the technique was not adopted due to the complexity

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CORRESPONDING AUTHOR: Joel Fernandez de Oliveira

Av. Dr. Arnaldo, 251, Cerqueira César, São Paulo, SP, Brasil – 01246-000

Tel: +55 11 3893-2000

E-mail: jfoliveira1@gmail.com

of the procedure. After the development of LAMS, Binmoeller and Shah<sup>5</sup> introduced the EUS-GE for the first time using LAMS in an animal model in 2012, thus enabling the expansion of the technique.

Traditionally, surgical gastrojejunostomy has been the main treatment for benign and malignant GOO, despite the high complication rate of up to 40%<sup>3,6</sup>. In addition, surgical treatment has some other limitations such as the prolonged recovery time that can delay the beginning of chemotherapy, gastroparesis, as well as the costs related to the procedure<sup>3</sup>.

In this study, we wanted to describe the results of our initial experience with EUS-guided gastroenterostomy.

## METHODS

This study was approved by CONEP (Comissão Nacional de Ética em Pesquisa) on October 1<sup>st</sup>, 2019.

Patients  $\geq 18$  years old, with a confirmed unresectable distal gastric, duodenal, or pancreaticobiliary malignancy, suffering from gastric outlet obstruction, with a gastric outlet obstruction score of  $\leq 1$ , and with a ECOG performance status  $\leq 3$  were eligible for inclusion. They were included in the study after signing the informed consent. The exclusion criteria included coagulation disorders, life expectancy shorter than 1 month, linitis plastica, previous gastric surgery, and radiological signs of small bowel obstruction.

There are a few different techniques of EUS-GE<sup>7</sup>. We adopted the technique that employs a double-balloon catheter, described by Itoi et al.<sup>8</sup>.

Initially, a guidewire was passed through the proximal jejunum, leaving a overtube in the antrum or duodenal bulb. A balloon occlusion catheter (Tokyo Medical University type, Create Medic Co., Ltd., Yokohama, Japan) was passed through the guidewire to the proximal small intestine. This new catheter has six radiopaque beads at the distal end and two balloons, separated by 20 cm, which when inflated fix a segment of the duodenum or jejunum. The segment of the small intestine between these two inflated balloons was then filled with contrast and methylene blue. Guided by EUS, a puncture of the distended small intestine between these two balloons was performed. The LAMS with an electrocautery-enhanced delivery system was directly implanted, creating the gastroenterostomy. The balloon catheter was then removed.

Patients were usually observed for 48 hours. Oral antibiotics (quinolone or a 3<sup>rd</sup> generation cephalosporin) were administered for another 7 days after the procedure.

A liquid diet was started the next day and was advanced to a low residue (soft) diet for the next 1-2 days, as tolerated. Stents were left in place indefinitely.

Patients had a follow-up visit 1 month after the procedure.

Technical success was considered when the adequate introduction of the LAMS communicating the stomach and the jejunum was achieved.

The ingestion of a soft diet 48h after the procedure was considered a clinical success.

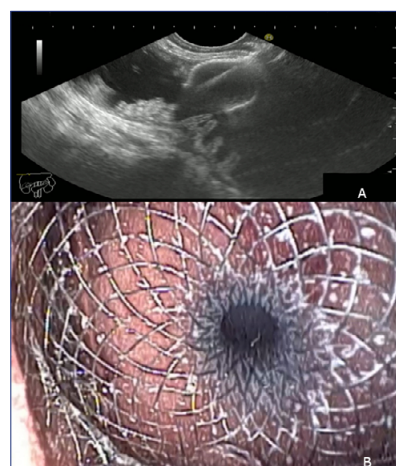
Adverse events included gastrointestinal bleeding (melena or hematemesis requiring blood transfusion or any invasive intervention), abdominal sepsis (clinical signs of infection, and peritonitis with leucocytosis and/or elevated reactive-C-protein).

## RESULTS

### Case 1

An 80-year-old female patient, in follow-up by a palatal neoplasm, had complained for two months of postprandial fullness and nausea. She underwent a upper GI endoscopy that showed a stenosing lesion on the transition between the duodenal bulb and the second duodenal portion. Computed tomography was performed, which showed an infiltrative lesion in the pancreatic head, causing a duodenal obstruction. Discussed with a multidisciplinary team and defined by the EUS-FNB (fine needle biopsy) EUS-GE with 15 mm Hot-axios™, both with technical success and confirmation of pancreatic adenocarcinoma. At follow-up after 3 months, she had a good acceptance of the regular diet and no procedure-related adverse events.

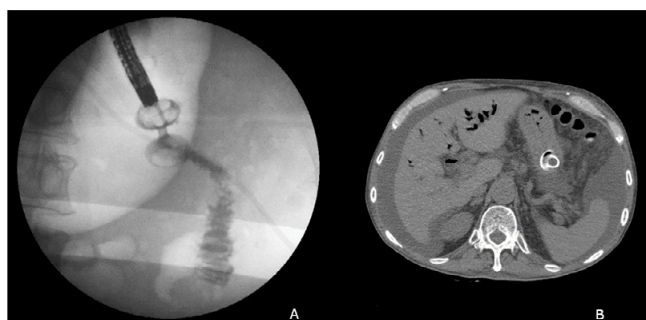
**FIGURE 1.** A: EUS VIEW OF THE LAMS RELEASE. B: ENDOSCOPIC VIEW OF THE LAMS



## Case 2

A 75-year-old male patient, with locally advanced pancreatic neoplasia who had undergone endoscopic introduction of a biliary metal prosthesis, was admitted to the emergency room with abdominal pain, nausea, and vomiting. Endoscopy demonstrated gastric stasis and a stenotic lesion in the duodenum. He was then submitted to EUS-GE with 15 mm Hot-axios™ with technical success. After 2 months, he reported good tolerance to a regular diet and no adverse events.

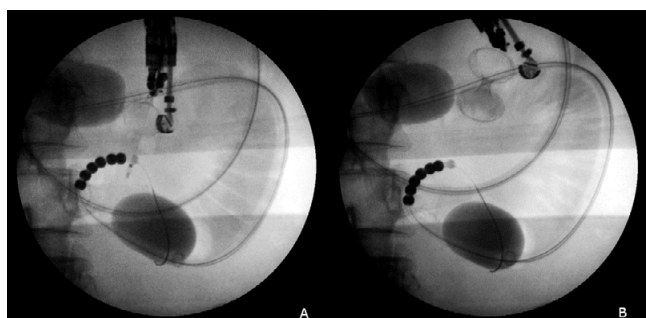
**FIGURE 2.** A: FLUOROSCOPY SHOWING GOOD PASSAGE OF CONTRAST FROM THE STOMACH TO THE JEJUNUM THROUGH THE LAMS. B: CT AFTER EUS-GE SHOWING THE LAMS



## Case 3

A 72-year-old female patient followed up for metastatic pancreatic neoplasia who had previously undergone hepatic-jejunal anastomosis was admitted to the emergency room with GOO. Endoscopy revealed a stenotic lesion in the second duodenal portion. She was submitted to EUS-GE with 20 mm Hot-axios™ with technical success. In the follow-up after 1 month, she presented good acceptance of a normal diet and no adverse events were observed.

**FIGURE 3.** A: INITIAL RELEASE OF LAMS. B: FINAL ASPECT OF EUS-GE



## DISCUSSION

This is the first Brazilian series describing the initial results of EUS-guided gastroenteroanastomosis in patients with GOO. In our study, we achieved technical and clinical success in all three patients. All patients were discharged 48 hours after the procedure with good tolerance to a low residue diet and evolution to a regular diet. It is noteworthy that all the patients were admitted with clear symptoms of GOO. In addition, we did not observe any procedure-related adverse events.

It is important to note that in the first two cases, we used a 15mm LAMS as the 20mm was not yet available in Brasil. However, despite the good evolution of patients with a 15mm-diameter stent, we believe that a 20 mm LAMS might offer results more similar to a surgical gastroenterostomy.

Initial outcomes of EUS-GE have been promising, regardless of technique, and for a variety of different indications. In two recent systematic reviews and meta-analysis, one with 5 studies including 199 patients (21% due to benign causes) and the second one with twelve studies including 285 patients, the reported technical success was 92% and clinical success, 90%. Adverse events occurred in 5 to 12%, and the re-intervention rate was 11%<sup>9,10</sup>.

In a study by Ge et al.<sup>11</sup> with 100 patients comparing EUS-GE and enteral stent placement in the palliation of malignant GOO, EUS-GE has a higher rate of initial clinical success (95.8% vs. 76.3%) and a lower rate of stent failure requiring repeat intervention (32.0% vs. 8.3%). The enteral stent group trended towards increased adverse events (40.2% vs. 20.8%,  $p = 0.098$ ). The mean length of hospital stay was similar between the groups<sup>11</sup>. Another study by Chen et al.<sup>12</sup> demonstrated a significantly lower risk of recurrence and reintervention in the EUS-GE group compared to the enteral stenting group (4.3% vs 28.6%).

In 2017, an international study compared EUS-GE versus laparoscopic gastrojejunostomy (Lap-GJ)<sup>13</sup>. The authors found a similar efficacy between the groups, although the EUS-GE group contained more complex patients. EUS-GE was cost-saving compared with Lap-GJ and caused less adverse events (12% vs 41%)<sup>13</sup>.

Another possibility of using EUS-GE is in the treatment of afferent loop syndrome. This consists of the obstruction of the afferent loop, with fluid pressure building up in the loop and causing discomfort and nausea. The incidence of this complication after pancreaticoduodenectomy may be as high as 13%<sup>14</sup>. A multicenter international experience on 18 patients

showed that EUS treatment seems to be safe and effective. An indirect comparison with enteroscopy-assisted luminal stenting suggested that EUS treatment is associated with a reduced need for re-intervention<sup>15</sup>.

Despite the encouraging results, some requirements must be met before considering EUS-GE. A target area of the duodenal or jejunal lumen must be located by EUS near (< 2cm) the gastric wall due to the risk of incomplete apposition of the lumens by the LAMS. Another important point is to exclude the possibility of malignant invasion of the stomach wall because of the risk of bleeding and incomplete apposition of the stent. Severe obstruction of the outlet resulting in the inability to advance a guidewire to the jejunum may render the procedure unfeasible. Ascites can also make the procedure difficult. Finally, care must be taken to ensure that there is no luminal obstruction downstream and distal to the puncture site of the jejunum, especially in cases of peritoneal carcinomatosis or extensive lymphadenopathy<sup>16,17</sup>.

## CONCLUSION

EUS-GE is a novel option for patients with GOO and our limited experience has demonstrated its feasibility and high clinical success. The results of retrospective studies suggest that EUS-GE may provide a more durable solution than enteral stenting and a less expensive solution than Lap-GJ with a shorter recovery time. Randomized controlled trials are warranted to support those views. In addition, EUS-GE is a technically challenging procedure that should only be carried out in centers with experience in therapeutic EUS.

## Author's Contribution

All authors contributed equally to the concept of the paper, and the draft was prepared by Joel Fernandez de Oliveira and the final review was completed by Fauze Maluf Filho.

## RESUMO

**INTRODUÇÃO:** A gastroenterostomia ecoguiada é um novo procedimento para palição da obstrução maligna gastroduodenal. Nosso objetivo foi avaliar os resultados dessa técnica em nossa experiência inicial.

**MÉTODOS:** Foram incluídos pacientes com obstrução maligna gastroduodenal de nossa instituição. O sucesso técnico foi definido como a realização adequada de uma gastroenterostomia. O sucesso clínico foi definido como boa aceitação de dieta pastosa durante a internação. Os eventos adversos e a aceitação alimentar foram avaliados um mês após o procedimento.

**RESULTADOS:** Três pacientes foram incluídos. Os sucessos técnico e clínico foram alcançados em todos os casos. Não houve eventos adversos e a aceitação alimentar permaneceu adequada um mês após o procedimento nos pacientes incluídos.

**CONCLUSÃO:** O EUS-GE é um tratamento promissor para pacientes com obstrução maligna gastroduodenal.

**PALAVRAS-CHAVE:** Endossonografia/métodos. Obstrução da saída gástrica. Gastroenterostomia/métodos. Ultrassonografia de intervenção/métodos.

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# Asymmetric parasitic twins - Heteropagus

 Rita de Cássia S. Alves<sup>1</sup>  
 Adriana Ubirajara Silva Petry<sup>1</sup>  
 Josenel Maria Barcelos Marçal<sup>1</sup>  
 Bruno Hochhegger<sup>1</sup>  
 Andréa Souto Damini<sup>2</sup>  
 Adriana Vial Roehe<sup>1</sup>

1. Docente, Departamento de Patologia e Medicina Legal, Universidade Federal de Ciências da Saúde de Porto Alegre (UFCSPA), Porto Alegre, RS, Brasil.

2. Docente, Departamento de Ginecologia e Obstetrícia, Universidade Federal do Rio Grande do Sul (UFRGS), Porto Alegre, RS, Brasil.

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## SUMMARY

**BACKGROUND:** *Asymmetric or heteropagus conjoined twins is a rare occurrence, with an incidence of one case in 1-2 million. Conjoined twins are classified according to their symmetry, place of fusion, and grade of duplication.*

**METHODS:** *We report here an extremely rare presentation of parasitic twins not described before. We describe macro and micro anatomic alterations and discuss the aspects of this peculiar presentation and the importance of prenatal diagnosis.*

**RESULTS:** *The case of a twenty-three-year-old patient, with monochorionic, monoamniotic asymmetrically-conjoined twin pregnancy, discovered at 29 weeks of gestational age. We believe that this report calls attention to this presentation and the importance of prenatal care and management. The twins were delivered vaginally without life. The twins' combined weight was 1.300 gr. They were bonded in the left cervical region.*

**CONCLUSION:** *This report may help to find strategies for clinical decisions in future cases. Antepartum diagnosis is important to the management, preoperative planning, and outcomes. Prenatal imaging exams like echocardiography, CT, MRI, and ultrasonography are feasible and can provide relevant information about malformation severity and prognosis.*

**KEYWORDS:** *Twins, Conjoined. Congenital abnormalities. Twins, Monozygotic.*

## INTRODUCTION

Conjoined twins are classified according to their symmetry, place of fusion, and grade of duplication, thoracopagus being the most frequent type (40%), followed by *thoracoomphalopagus* (28%). Asymmetrically conjoined twins are also known as parasitic twins or *heteropagus*, and usually, they do not fit well in any classification<sup>1</sup>.

Asymmetric or *heteropagus* conjoined twins are defined by Spencer as one severely damaged twin

attached to a relatively normal twin through an asymmetric anatomical location<sup>2</sup>. One twin of the pair (autosite) is mostly intact, even though some congenital abnormalities can be observed. Its counterpart, the one called the parasite is badly defective and depends on the cardiovascular system of the autosite for survival<sup>3</sup>. It is an extremely rare occurrence, with an incidence of one case in 1 - 2 million births<sup>4,5</sup>.

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CORRESPONDING AUTHOR: Adriana Vial Roehe

Rua Sarmento Leite, 245, Porto Alegre, RS, Brasil – 90050-170

Tel: +55 51 3033-8723 / Fax: +55 51 3303-8810

E-mail: aroehe@gmail.com; adrianar@ufcspa.edu.br



## METHODS

A twenty-three-year-old patient, P2L1, with no contributive medical history, went to the emergency service of the Femina Hospital, Porto Alegre, Brasil, with abdominal pain, in labor. The patient did not know about her pregnancy, but a monochorionic, mono-amniotic conjoined twin pregnancy was discovered (29 weeks of gestational age) in a emergency ultrasonography examination. Intrauterine death was diagnosed and the fetuses were delivered vaginally without dystocia and induction; they proved to be conjoined twins with no cardiac activity.

## RESULTS

The twins' combined weight was 1.300 gr. The autosite fetus crown-heel length was 27.5 cm crown-rump length was 15.5 cm and head circumference was 15.6 cm. The gestational age was estimated at 29 weeks.

An ectoscopic examination demonstrated the parasite twin was bonded to the left cervical region of the autosite (Fig. 1).

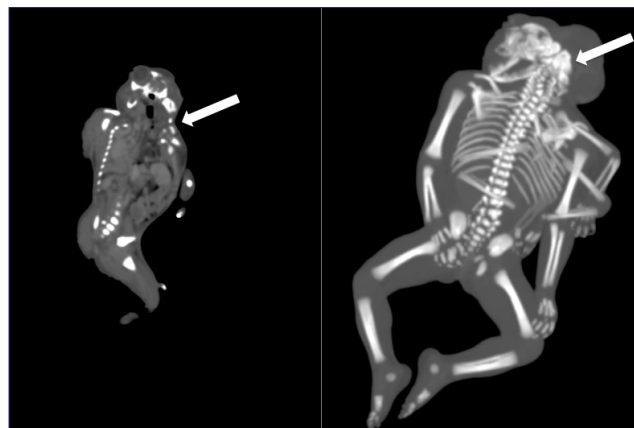
**FIGURE 1.** MACROSCOPIC FRONTAL VIEW OF THE PARASITIC TWINS.



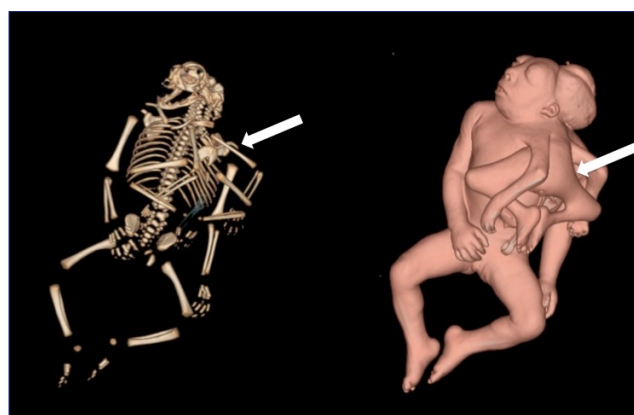
There were relatively well-formed upper and lower limbs. The parasite also had a rudimentary pelvis, with external female genitals and anus. CT scan features can be observed in Fig. 2 and 3.

*Post mortem* analysis of the parasite fetus showed pelvic organs inside the autosite fetus chest cavity. There was a rudimentary bladder and one kidney. These tissues were taken for histopathological examination. There was also a single intestinal loop from the parasite fetus fused with the intestinal loops of the autosite. No thoracic structures were observed in the parasite fetus.

**FIGURE 2.** (A) CORONAL CT SCAN AND (B) MINIMUM INTENSITY PROJECTION CORONAL CT SCAN OF THE PARASITIC FETUS REVEALS A HETEROGENEOUS MASS (ARROW) IN THE CERVICAL REGION WITH BONY COMPONENTS. THE BONY COMPONENTS (ARROW) RESEMBLE A VERTEBRAL BODY IN CROSS-SECTION. ALSO, CT DEMONSTRATED ANENCEPHALY.



**FIGURE 3.** (A) VOLUME RENDERING BONE PROJECTION CT SCAN AND (B) VOLUME RENDERING SKIN PROJECTION CT SCAN OF THE PARASITIC FETUS DEMONSTRATED A HETEROGENEOUS MASS AT THE LEVEL OF THE AXILLA (ARROW) THAT CONSISTS OF AN OSSEOUS COMPONENT RESEMBLING THE PELVIS AND LUMBOSACRAL SPINE.



The autosite fetus was anencephalic. There were no facial or cervical malformations. The thoracic organs were topic. The lungs were hypoplastic and parts of the intestinal loops were located in the chest cavity (Fig. 6 of the Supplementary File). The other abdominal organs were topic and no abnormality was observed.

## DISCUSSION

Conjoined twins are classified according to their symmetry, place of fusion, and grade of duplication. Different from the usual conjoined twins that have the same sites fused, parasitic twins are a subset in which "asymmetric" joining occurs, having their

own variants and independent classification. In this case report, the parasite twin was attached to the left lateral thoracervical region of the autosite, in an extremely rare presentation, not classified and not described previously<sup>3</sup>.

According to Sharma et al.<sup>3</sup>, there can be a single heart, single liver, small bowel loops crisscrossed from one fetus to the other. Other authors describe some cases of urinary tract communication between the parasite and autosite fetus<sup>3</sup>. Some cases of urinary tract communication between the parasite and autosite fetus have also been described<sup>d3</sup>. In our case, the parasite fetus has a rudimentary urinary system without communication with the autosite urinary system. The parasite fetus had a single intestinal loop connected to autosite intestinal loops. This feature is described in 28% of parasitic fetus<sup>3</sup>.

Anencephaly is described more frequently in *parapagus* and *dicephalus* twins, and most *heteropagus* parasite twins are anencephalic<sup>1,3</sup>. The autosite fetus reported here was also anencephalic. There was an amorphous mass of fibroangiomatous tissue in the occipital and cervical region between the autosite and parasitic fetus, probably corresponding to an anencephaly of the parasitic fetus as well. According to the CT scan, there was cervical spinal duplication. Except for anencephaly, there was no other significant malformation in the autosite fetus. In many cases of *heteropagus* twins, the prognosis is determined by cardiac abnormalities. In this case, the autosite fetus did not have any cardiac malformation.

As in our case, none of the reviewed cases presented a history of consanguinity and most of them did not have significant pregnancy complications. The etiology of conjoined twins is still unknown and

the pathogenesis is obscure. Some studies refer to a discreet prevalence of female twins, while gender differences are less pronounced in *heteropagus* twins<sup>2,3</sup>.

In this case report, the mother did not know about her pregnancy and had not made any previous visits to a doctor. Frequently, the prenatal diagnosis of this condition is made between 9 and 28 weeks. Spontaneous abortion is observed in 12% of the cases of *heteropagus* twins. In our case, the abortion occurred lately<sup>3</sup>.

Before the widespread use of ultrasound, most conjoined twins could not be identified intra-uterus. Imaging diagnosis advances have improved the approach to conjoined twins. Exact and detailed prenatal diagnosis of conjoined twins is possible and essential for optimal obstetric management and parental counseling.

## CONCLUSION

In conclusion, as each set of conjoined twins is unique, the description of each new presentation is contributive to correlate and to guide imaging diagnosis. Imaging exams play an important role in proposing medical conduct, mostly because asymmetric *heteropagus* twins have less extensive vascular and visceral connections, determining a better prognosis to autosite fetus.

## Author's Contribution

Rita de Cássia Sant'Anna Alves, Adriana Ubirajara Silva Petry, Andréa Pires Souto Damin – literature review and text formatting; Josenel Maria Barcelos Marçal, Adriana Vial Roehe – necropsy performance and text formatting; Bruno Hochhegger – image examinations.

## RESUMO

**BACKGROUND:** Gêmeos parasitas assimétricos ou heterópagos são uma ocorrência rara, com incidência de um caso em 1-2 milhões. Os gêmeos siameses são classificados de acordo com sua simetria, local de fusão e grau de duplicação.

**MÉTODOS:** Relatamos aqui uma apresentação extremamente rara de gêmeos parasitários não descritos anteriormente. São descritas alterações macro e microscópicas e discutidos aspectos relevantes dessa malformação e da importância do diagnóstico pré-natal.

**RESULTADOS:** Um caso de uma paciente de 23 anos de idade, com gestação monócórionica, monoamniótica de gêmeos siameses assimétricos diagnosticada com 29 semanas de idade gestacional. Acreditamos que este relato chama a atenção para esta apresentação e para a importância do cuidado e manejo pré-natal. Os fetos nasceram de parto vaginal já sem vida, pesando em conjunto 1.300 gramas, e eram unidos pela região cervical esquerda.

**CONCLUSÃO:** Este relato pode ajudar a encontrar estratégias para a decisão clínica em casos futuros. O diagnóstico pré-natal é fundamental para o manejo e planejamento pré-operatório. Exames de imagem como ecocardiografia, tomografia computadorizada, ressonância magnética e ultrassonografia são factíveis e podem fornecer informações-chave sobre a gravidade e prognóstico da malformação.

**PALAVRAS-CHAVE:** Gêmeos unidos. Anormalidades congênitas. Gêmeos monozigóticos.

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# High-expression of LncRNA MAFG-AS1 is associated with the prognostic of patients with colorectal cancer

 Weichun Cui<sup>1</sup>  
 Yong Wang<sup>1</sup>  
 Xiangji Shen<sup>2</sup>  
 Xudong Wu<sup>1</sup>  
 Hui Liu<sup>1</sup>  
 Xiuhua Xu<sup>1</sup>

**1.** Department of Gastrointestinal Surgery, Dongying People's Hospital, Dongying 257091, Shandong, China  
**2.** Department of General Surgery, The People's Hospital of Guangrao, Guangrao 257000, Shandong, China

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## SUMMARY

**OBJECTIVE:** Long noncoding RNAs (lncRNAs) have been proven to exhibit distinct functions on the convoluted processes of tumor developments. Some studies on the biological functions of lncRNA MAFG-AS1 (MAFG-AS1) in cancers revealed that they may serve as an oncogene in some kinds of tumors, including colorectal cancer (CRC). However, little is known about the role of MAFG-AS1 in the prognostic of CRC.

**METHODS:** A public dataset was mined for the screening of dysregulated lncRNAs in CRC. Quantitative real-time reverse transcription-polymerase chain reaction (qPCR) was used to compare the levels of MAFG-AS1 between paired MAFG-AS1 specimens and normal adjacent tissues. The correlations between MAFG-AS1 and clinic pathological features in CRC were analyzed using the chi-square test. The log-rank test and Kaplan-Meier test were carried out to compare the survival time of patients with high and low expressions of MAFG-AS1. Cox regression was applied for univariate and multivariate assays to validate whether MAFG-AS1 could be an independent factor in the prognosis of CRC.

**RESULTS:** We found that the distinct upregulation of MAFG-AS1 in various tumors was a common event. MAFG-AS1 was distinctly up-regulated in CRC specimens compared to matched non-tumor specimens ( $p < 0.01$ ). High MAFG-AS1 expressions were closely associated with depth of invasion ( $p = 0.011$ ) and TNM stage ( $p = 0.022$ ). Survival assays revealed that patients with high expression of MAFG-AS1 have a shorter overall survival ( $p = 0.0030$ ) and disease-free survival ( $p = 0.0002$ ). **CONCLUSIONS:** MAFG-AS1 can serve as a novel potential biomarker to predict CRC patients' survival time.

**KEYWORDS:** RNA, long noncoding. Colorectal neoplasms. Survival analysis.

## INTRODUCTION

Colorectal cancer (CRC) is one of the most frequent cancers in human beings, accounting for 762,400 deaths annually worldwide<sup>1-3</sup>. In recent years, the incidence and mortality rates of CRC have been evaluated to increase gradually in many countries,

especially in several low-income countries<sup>4</sup>. Despite the development of chemotherapeutic and surgical therapies, coupled with the advancements of novel molecular targeted therapeutics, have improved the five-year survival rate of CRC patients, the long-term

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CORRESPONDING AUTHOR: Xiuhua Xu

Department of Gastrointestinal Surgery, Dongying People's Hospital, No. 317 Nanyi Road, Dongying District, Dongying, Shandong 257091 – China. Tel/ Fax: +86- 546-8901206

Email: xiuhua257091@163.com

survival of patients with advanced CRC remains poor<sup>5,6</sup>. The probabilities of recurrence and metastasis are distinctly increased once the neoplasms enter the advanced stages, which is the main cause for the death of patients<sup>7</sup>. Thus, further knowledge of the potential mechanisms of CRC developments and an assessment of novel biological markers are required for the early detection and treatment of CRC.

Long non-coding RNAs (lncRNAs) are a set of RNAs with > 200 nucleotides in size and have limited protein-coding abilities due to the lack of an open read frame<sup>8</sup>. Recently, the imperative effects of lncRNAs in the modulation of gene expression have been frequently reported in a large number of studies, involved in the epigenetic regulation<sup>9,10</sup>. Without a doubt, various biological progressions, such as chromosome replication, cytodifferentiation, life-cell metastasis and apoptosis, were influenced by the dysregulation of lncRNAs<sup>11,12</sup>. With the development of sequencing technology and bioinformatics, growing abnormally-expressed protein<sup>13</sup> and lncRNAs have been periodically observed in various tumors involved in carcinogenesis and tumor progression, suggesting that several lncRNAs could function as novel biomarkers for early diagnosis, prognosis prediction, and sufficient therapeutic targets of malignancies<sup>14,15</sup>. However, the investigation of further CRC-associated lncRNAs is warranted.

MAFG-AS1, a novel tumor-related lncRNA, has been reported to be abnormally expressed in several tumors, including CRC, breast carcinoma, and lung adenocarcinoma<sup>16-18</sup>. However, the potential function of MAFG-AS1 in tumors remains largely unclear. Recently, Cui et al.<sup>16</sup> firstly reported MAFG-AS1 was an oncogenic lncRNA in CRC due to its frequent upregulation in CRC tissues and tumor-promotive roles in the proliferation and metastasis of CRC cells. However, the relative evidence supporting the over-expression of MAFG-AS1 was limited. In addition, its clinical significance in CRC patients remains to be further investigated.

## METHODS

### Patients and Tissue Samples

A total of 172 pairs of fresh CRC and adjacent non-tumor specimens were obtained from 172 patients with CRC, who underwent clinical surgery at our Hospital, Dongying People's Hospital, between April 2011 and July 2014. This cohort consisted of 72 females

and 100 males aged from 31 to 71 years (mean age was 43.7). All of the cases were histopathologically diagnosed as rectum carcinomas by two independent pathologists from our hospital. None of these patients had received radiotherapy or chemotherapy before surgery. To track the differential survivals, a five-year follow-up was conducted by straightforward telephone interviews or external checks until death or metastasis. The study protocol was approved by the ethics committee of the Dongying People's Hospital, and informed consent was obtained from all the patients.

### RNA extraction and quantitative real-time reverse transcription-polymerase chain reaction (qPCR)

Total RNA from CRC specimens and matched normal samples were extracted using TRIzol reagent (Ming Technology, Hangzhou, Zhejiang, China) according to the company's handbook. The purity quotient of RNAs was examined using agarose gel electrophoresis, followed by DNAase for the elimination of impurities. A RevertAid™ First-Strand cDNA Synthesis Kit was purchased from Bichennan Biotechnology (Haidian, Beijing, China) and used to synthesize first-strand complementary DNA (cDNA) using a random primer. Then, an ABI Prism 7500 (Biosystems, Pudong, Shanghai, China) was used for the qRT-PCR assays, which were performed using a TaqMan MicroRNA Assay Kit (Biosystems, Pudong, Shanghai, China). The expression levels of MAFG-AS1 were analyzed by applying the comparative cycle threshold (CT) ( $2^{-\Delta\Delta CT}$ ) methods with GAPDH as the endogenous control. The sequences of the PCR primers used in this study were as follows: MAFG-AS1 5'-ATGACGACCCCAATA-AAGGG-3' (sense); 5'-CACCGACATGGTTACCAGC-3' (antisense). GAPDH: 5'-GAAGGTGAAGGTCGGAGT-3' (sense); 5'-GAAGATGGTGACTGGGATTT-3' (antisense).

### Statistical analysis

All data were analyzed using the SPSS 19.0 software (SPSS Inc., Chicago, IL, USA). The associations between the MAFG-AS1 levels and clinicopathological factors were analyzed applying the chi-square test. The Student's *t*-test was applied to determine the statistical significance of the difference between CRC tissues and matched non-tumor specimens. The disease-free survival (DFS) and overall survival (OS) curves were plotted using the Kaplan-Meier methods, followed by the log-rank test to evaluate the statistical significances. Univariate and multivariate assays were



further used to explore the independent prognostic factors for CRC. Results were considered statistically significant at  $p < 0.05$ .

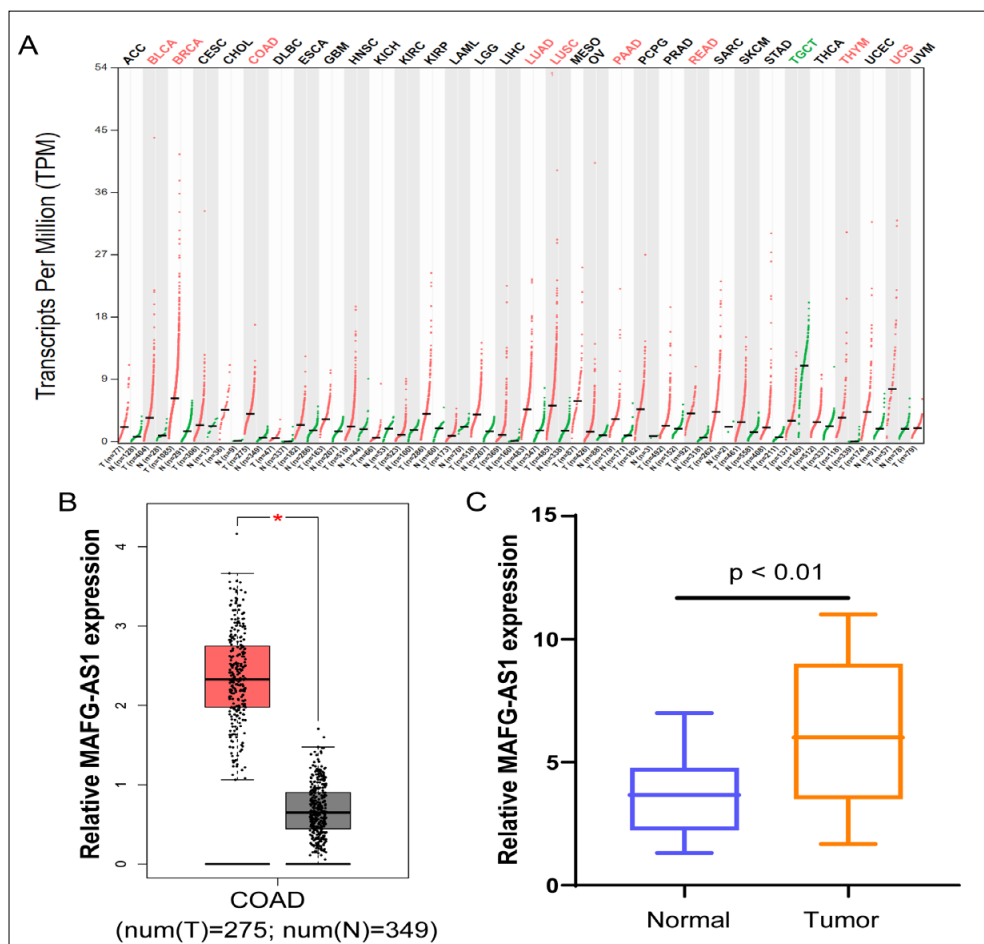
## RESULTS

### Increased expression of MAFG-AS1 in human CRC

The clinicopathological parameters of all 172 cases are presented in Table 1. To study the expression pattern of MAFG-AS1 in tumors, we searched an online system, the Gene Expression Profiling Interactive Analysis (GEPIA), which can be used to analyze the microarray data of gene expressions from TCGA datasets. As shown in Figure 1A, the distinct upregulation of MAFG-AS1 was observed in several different tumors, including CRC. Specifically, the expressions of MAFG-AS1 in 275 CRC specimens were distinctly higher than those in 349 non-tumor specimens ( $p < 0.01$ ). In order to validate the above findings, we performed a RT-PCR to examine the levels of MAFG-AS1 in our cohort, further confirming the distinct overexpression of MAFG-AS1 in CRC tissues ( $p < 0.01$ ).

**TABLE 1.** CORRELATION BETWEEN CLINICOPATHOLOGIC CHARACTERISTICS AND THE EXPRESSION OF MAFG-AS1 IN CRC.

| Clinicopathological features | No. of cases | MAFG-AS1 expression |     | p-value |
|------------------------------|--------------|---------------------|-----|---------|
|                              |              | High                | Low |         |
| Age                          |              |                     |     |         |
| <55                          | 88           | 40                  | 48  | 0.287   |
| ≥55                          | 84           | 45                  | 39  |         |
| Gender                       |              |                     |     | 0.268   |
| Male                         | 100          | 53                  | 47  |         |
| Female                       | 72           | 32                  | 40  |         |
| Tumor location               |              |                     |     | 0.886   |
| Colon                        | 91           | 47                  | 44  |         |
| Rectum                       | 81           | 38                  | 34  |         |
| Tumor size                   |              |                     |     | 0.093   |
| <5cm                         | 102          | 45                  | 57  |         |
| ≥5cm                         | 70           | 40                  | 30  |         |
| Histology/differentiation    |              |                     |     | 0.065   |
| Well + Moderate              | 105          | 46                  | 59  |         |
| Poor                         | 67           | 39                  | 28  |         |
| Depth of invasion            |              |                     |     | 0.011   |
| T1 + T2                      | 117          | 50                  | 67  |         |
| T3 + T4                      | 55           | 35                  | 20  |         |
| TNM stage                    |              |                     |     | 0.022   |
| I-II                         | 123          | 54                  | 69  |         |
| III                          | 49           | 31                  | 18  |         |



**FIGURE 1.** MAFG-AS1 EXPRESSION WAS UP-REGULATED IN CRC TISSUES



## Clinical and pathological significance of MAFG-AS1 expression in CRC

To study the significance of MAFG-AS1 levels in the clinical progress of CRC patients, our group divided 172 patients into two groups: A high MAFG-AS1 expression group,  $n = 85$ , and a low MAFG-AS1 expression group,  $n = 87$ . Then, the chi-square test was applied to analyze the clinical data. Interestingly, in our cohort, high levels of MAFG-AS1 were positively associated with depth of invasion ( $p = 0.011$ ) and TNM stage ( $p = 0.022$ ). However, no distinct associations between MAFG-AS1 expressions and other clinical and pathological parameters were observed ( $p > 0.05$ ).

### Positive associations between MAFG-AS1 expressions and prognostic values of CRC patients

Given that the dysregulation of MAFG-AS1 was positively correlated with tumor invasion and advanced stages, we speculated that the levels of MAFG-AS1 may influence the long-term survival of CRC patients. The results of the Kaplan-Meier assays revealed that patients with high expressions of MAFG-AS1 had a shorter OS ( $p = 0.0030$ ; Figure 2A) and DFS ( $p = 0.0002$ ; Figure 2B) as compared with the low-MAFG-AS1 group. Further univariate analysis indicated that depth of invasion, TNM stage, and MAFG-AS1 expression were distinctly associated with the OS and DFS of CRC patients (all  $p > 0.05$ ). More importantly, multivariate assays for the above three factors demonstrated that MAFG-AS1 expression, in addition to depth of invasion and TNM

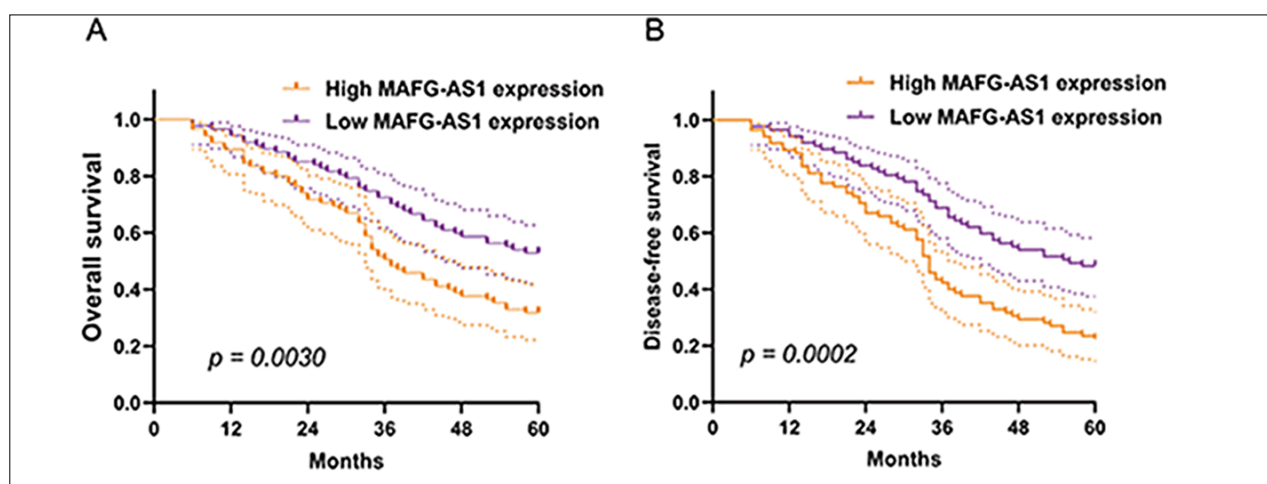
stage, were independent prognostic factors for both the OS (HR=2.737, 95% CI: 1.185-4.386,  $p = 0.021$ ) and DFS (HR=2.869, 95% CI: 1.185-4.562,  $p = 0.013$ ) of CRC patients.

## DISCUSSION

In recent years, basic studies have highlighted the critical effects of lncRNAs in the progression of diseases, including tumors<sup>19,20</sup>. In addition, more and more chip databases, such as the TCGA, GEO and COSMIC, displayed a large number of dysregulated lncRNAs in various types of tumors<sup>21,22</sup>. On the other hand, the clinical values of lncRNAs acting as independent prognostic biomarkers have also been frequently reported. In this study, our attention was focused on a novel CRC-related lncRNA.

It is well known that lncRNAs act as imperative regulators in tumorigenesis by regulating numerous cellular biological phenotypes, such as cellular growth, invasive abilities, chemo-radioresistance, and so on<sup>23</sup>. Recently, the dysregulation of MAFG-AS1 expression and its functional effects in several tumors have been reported in a few studies. For instance, the distinct overexpression of MAFG-AS1 was observed in breast cancer, and its knockdown was found to suppress the migration and invasion of breast cancer cells via the modulation of miRNA-339-5p/MMP15<sup>17</sup>. In lung cancer, a similar expression trend of MAFG-AS1 was also found in lung cancer. Functionally, the overexpression of MAFG-AS1 may result in increased abilities of migration and invasion in lung cancer cells by regulating the miRNA-339-5p/

**FIGURE 2.** SURVIVAL ANALYSIS OF 172 MAFG-AS1 PATIENTS BY KAPLAN-MEIER.



MMP15 axis<sup>18</sup>. A recent study by Ouyang et al.<sup>24</sup> indicated that the levels of MAFG-AS1 were increased in hepatocellular carcinoma specimens and cell lines. In their *in vitro* assays, the knockdown of MAFG-AS1 was demonstrated to inhibit the proliferation and metastasis of hepatocellular carcinoma cells via sponging miRNA-6852. Importantly, in CRC, MAFG-AS1 expression was reported to be upregulated, and its oncogenic roles in CRC proliferation and metastasis by modulating miR-147b/NDUFA4 were also observed<sup>16</sup>. Although MAFG-AS1 was also found to predict advanced clinical stages in CRC patients, the prognostic values of MAFG-AS1 in CRC patients have not been investigated.

In this study, we further explored the expression pattern of MAFG-AS1 in various tumors using “GEPIA” and found that the overexpression of MAFG-AS1 in tumor specimens was a common event, which highlighted the potential regulatory function of MAFG-AS1 in tumor progression. In our cohort, significantly increased levels of MAFG-AS1 were also observed. A previous study by Cui et al.<sup>16</sup> also provided evidence that MAFG-AS1 was highly expressed in both CRC specimens and cell lines, which was consistent with our findings. Then, we analyzed the clinical and pathological data to study the clinical significance of MAFG-AS1 expression in CRC patients, finding that a high expression of MAFG-AS1 was strongly associated with depth of invasion and TNM stage, which was also in line with

previous findings that forced MAFG-AS1 expression could promote the metastasis of CRC cells<sup>16</sup>. Moreover, we first performed Kaplan-Meier assays to analyze the survival data with five years of follow-up and found that the 5-year OS and DFS of the high MAFG-AS1 expression group were distinctly shorter than those of the low MAFG-AS1 expression group. Further multivariate assays using the Cox regression model confirmed that a high MAFG-AS1 expression was an independent prognostic factor for CRC. However, given the small number of patients in our clinical assays, further research using a great number of CRC patients is required to further validate our findings.

## CONCLUSIONS

Our findings initially suggested that MAFG-AS1 could serve as a potential target for treatment intervention and as a novel biomarker for CRC.

## Conflict of Interest

The authors declare that there are no conflicts of interest.

## Author's Contribution

All authors contributed to the data analysis, drafting and revising of the article, gave final approval of the version to be published, and agree to be accountable for all aspects of the work.

## RESUMO

**OBJETIVO:** RNAs longos não codificantes (lncRNAs) comprovadamente apresentam funções distintas no complicado processo de desenvolvimento de tumores. Alguns estudos sobre as funções biológicas dos lncRNA (MAFG-AS1) em cânceres revelaram que eles podem funcionar como um oncogene em alguns tipos de tumores, inclusive no câncer colorretal (CCR). No entanto, pouco é conhecido sobre o papel do MAFG-AS1 no prognóstico do CCR.

**MÉTODOS:** Um conjunto de dados públicos foi analisado em busca de lncRNAs desregulados em casos de CCR. Uma análise quantitativa de reação em cadeia de polimerase precedida de transcrição reversa (qPCR) em tempo real foi utilizada para comparar os níveis de MAFG-AS1 entre pares de amostras de MAFG-AS1 normal e tecidos adjacentes. As correlações entre MAFG-AS1 e características clínico-patológicas do CCR foram analisadas através do teste qui-quadrado. Os testes de Log-rank e Kaplan-Meier foram utilizados para comparar o tempo de sobrevida de pacientes com expressões altas e baixas do MAFG-AS1. A regressão de Cox foi aplicada para ensaios uni e multivariados para validar se MAFG-AS1 poderia ser um fator independente no prognóstico do CCR.

**RESULTADOS:** Observamos que a regulação aumentada de MAFG-AS1 em vários tumores foi um evento comum. MAFG1 estava claramente com a regulação aumentada em espécimes de CCR quando comparado ao de espécimes equivalentes não-tumorais ( $p < 0,01$ ). Altas expressões de MAFG-AS1 estavam associadas à profundidade de invasão ( $p = 0,011$ ) e classificação no sistema TNM ( $p = 0,022$ ). As análises de sobrevida revelaram que pacientes com expressões elevadas do MAFG-AS1 tiveram uma diminuição significativa da sobrevida global ( $p = 0,0030$ ) e da sobrevida livre de doença ( $p = 0,0002$ ). **CONCLUSÃO:** MAFG-AS1 pode ser um novo potencial biomarcador para prever o tempo de sobrevida de pacientes de CCR.

**PALAVRAS-CHAVE:** RNA longo não codificante. Neoplasias colorretais. Análise de sobrevida.

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# Triathlon-related musculoskeletal injuries: a study on a Portuguese Triathlon Championship

 Beatriz Minghelli<sup>1,2</sup>  
 Catarina Jesus<sup>1</sup>  
 Ilvanda Martins<sup>1</sup>  
 Joana Jesus<sup>1</sup>

1. School of Health Jean Piaget Algarve – Piaget Institute – Enxerim, Portugal  
 2. Research in Education and Community Intervention (RECI); Silves, Portugal

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## SUMMARY

**BACKGROUND:** A triathlon consists of 3 modalities: swimming, cycling and running. Its higher training frequency, the environmental factors and extrinsic factors (technique and equipment) may lead to injuries. This study aimed to determine injury epidemiology in triathletes, as well as their type, location, mechanism of injury, and risk factors.

**METHODS:** The sample was constituted of 174 triathletes who participated in the Triathlon Club Portuguese Championship, being 131 (75.3%) male, aged 18 to 70 years old (36.09±11.03). The measurement instrument was a questionnaire concerning the characterization of the population and aspects related to the modality and injuries.

**RESULTS:** One hundred and twenty (69%) athletes reported one injury since the beginning of their practice, and 95 (54.6%) had an injury in the previous year, with a total of 130 injuries. There were 2.39 injuries per 1,000 hours of triathlon training. The most common injuries were muscle contusion (31.5%) and inflammatory injuries (19.2%), located in the knee (22.3%) and the leg (18.5%). Overtraining (43.1%) was the mechanism that led to the highest occurrence of injuries. Fourteen (10.8%) injuries occurred during swimming, 23 (17.7%) during cycling, and 93 (71.5%) during running. No statistical significance was observed between the risk factors analyzed and the occurrence of injuries.

**CONCLUSIONS:** Triathlon practice is associated with a high prevalence of injuries, being contusions, knee, and overtraining the most common type, location, and mechanism of injury respectively. It is necessary to create injury prevention strategies, including specific training and suitable materials for use by athletes.

**KEYWORDS:** Bicycling/injuries. Running/injuries. Swimming/injuries. Epidemiology. Prevalence. Wounds and Injuries.

## INTRODUCTION

Triathlon includes swimming, cycling and running, which are performed consecutively in that order<sup>1</sup>. The most common formats for triathlon competitions are Short Distance (Sprint and Olympic) and Long Distance Triathlon (Half-Ironman and Ironman)<sup>2,3</sup>.

The number of triathletes and their performance level has increased. The Portuguese Federation of

Triathlon had 3,100 affiliated athletes and 115 associated clubs in 2018, demonstrating a huge growth of the sport in the country<sup>4</sup>.

Triathlon competitions are performed outdoors, thus several factors may predispose to injuries, such as the weather conditions of some competitions, hyperthermia, and other heat-related illnesses marked

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CORRESPONDING AUTHOR: Beatriz Minghelli

Instituto Piaget - Escola Superior de Saúde Jean Piaget / Algarve – Enxerim – 8300-025 – Silves – Portugal

Tel.: 00 (351) 282 440 170

Email: beatriz.minghelli@silves.ipiaget.pt

by collapse, followed by severe muscle cramping that impedes the athlete to continue the activity because of fatigue installation<sup>1</sup>. Besides that, a higher training frequency can also cause injuries. Triathletes train for more hours on average than single-sport athletes, averaging 10 to 14 hours, 8 to 10 times of training per week, which leads to a higher incidence of injury<sup>1,5</sup>.

The poorer technique, which leads to training errors, and inadequate equipment are considered to be the most common extrinsic causes of injuries<sup>1</sup>.

Overall, there is a limited number of investigations focusing on triathlon-related injuries, thus this study aimed to determine injury epidemiology in triathletes, as well as their type, location, mechanism of injury, and risk factors.

## METHODS

A cross-sectional study was performed to determine the epidemiology of musculoskeletal injuries in triathletes. The study was approved by the Piaget Institute's Research Unit RECI - Research in Education and Community Intervention.

### Population

The population was composed of triathletes who participated in the "Triathlon Club National Championship" (Olympic Triathlon format) held in the South of Portugal.

The sample size was determined using an estimated prevalence of injuries of 50%, a population of 321 triathletes who participated in this championship (259 male and 62 female), and assuming an error margin of 5% with a 95% confidence interval (CI). Using these assumptions, the minimum sample size was 173 triathletes.

The inclusion criteria involved triathletes of both sexes and any age, who had been training for at least six months, who trained at least 2 times per week, and who freely agreed to participate in the research and thus duly signed the informed consent form.

### Measurement instrument

The measurement instrument consisted of a structured questionnaire applied as an interview form by evaluators, in a single moment.

Since measurement instruments for assessing the presence of injuries in triathletes are unknown, this questionnaire was developed by the researchers and subsequently assessed by a panel of experts. In

addition, a pre-test was performed on 10 athletes to understand the difficulties of understanding and to measure the time of completion.

The questionnaire consisted of 2 parts: 1. questions about the socio-demographic characterization of the population and modality, and 2. specific questions about injuries related to triathlon practice.

The athletes who presented an injury in the previous 12 months were asked to continue to fill in the questionnaire regarding the injury characteristics. The interviewees were allowed to specify the characteristics of a maximum of three injuries (those considered most serious and/or needed more time for recovery) for all sports (swimming, cycling and running).

### Statistical analysis

Descriptive statistical data were obtained regarding all the variables in the study. Injury proportion (IP) and injury rate (IR) were calculated.

The influence of the included variables on the presence of injury in the previous 12 months was assessed using binary logistic regressions, based on the Enter methods, and crude odds ratios, and the corresponding confidence intervals (CI) were calculated. Statistical significance was set at 0.05.

Data analysis was performed with Statistical Package for Social Sciences (SPSS), version 24.0.

## RESULTS

The sample was constituted by 174 triathletes, being 131 (75.3%) male and 43 (24.7%) female athletes, ages between 18 and 70 years old ( $36.09 \pm 11.03$ ).

Regarding the years of triathlon practice, most athletes practiced the sport between 3 and 5 years (56; 32.2%), 42 (24.2%) for 10 years or more, 31 (17.8%) between 6 and 7 years, 27 (15.5%) between 1 and 2 years, 16 (9.2%) between 8 and 9 years, and 2 (1.1%) between 6 to 11 months.

Most of the athletes trained every day (92; 52.9%), 33 (19%) trained six times a week, 25 (14.4%) trained 5 times a week, 11 (6.3%) four times, 7 (4%) three times, 3 (1.7%) twice a week, and 3 (1.7%) trained only once a week. One-hundred-and-thirty (74.7%) athletes trained between 1 to 2 hours, 38 (21.9%) for 3 hours or more, and 6 (3.4%) up to 45 minutes per session.

One-hundred-and-fifty-six (89.7%) athletes reported performing some type of warm-up before training or competing, and 140 (80.5%) performed a cool-down.

One-Hundred-and-one (58%) athletes participated



in the Sprint modality with more frequency, 22 (12.6%) in Olympic, 48 (27.6%) in Half-Ironman, and only 3 (1.7%) in Ironman competition.

One-hundred-and-twenty (69%) athletes reported having suffered from some type of injury since the beginning of their triathlon practice, 29 (16.7%) were injured at the day of competition, 74 (42.5%) referred having injuries in a 6-month period.

Ninety-five (54.6%) athletes referred to having injuries in a 12-month period. Of these athletes (95; 100%), 63 (66.3%) reported one injury, 29 (30.5%) two injuries, 3 (3.2%) three injuries. Of those, 14 (10.8%) occurred during swimming, 23 (17.7%) during cycling, and 93 (71.5%) during running, totaling 130 injuries.

The average number of injuries per athlete (total number of injuries/total number of athletes) was 0.74, and the average of injuries per injured athlete (total number of injuries/total number of injured athletes) was 1.36.

An injury proportion of 0.55 (CI 95%: 0.05-0.09) injuries per athlete, per 12 months, was obtained. The injury rate was 2.39 injuries per 1,000 hours of triathlon training.

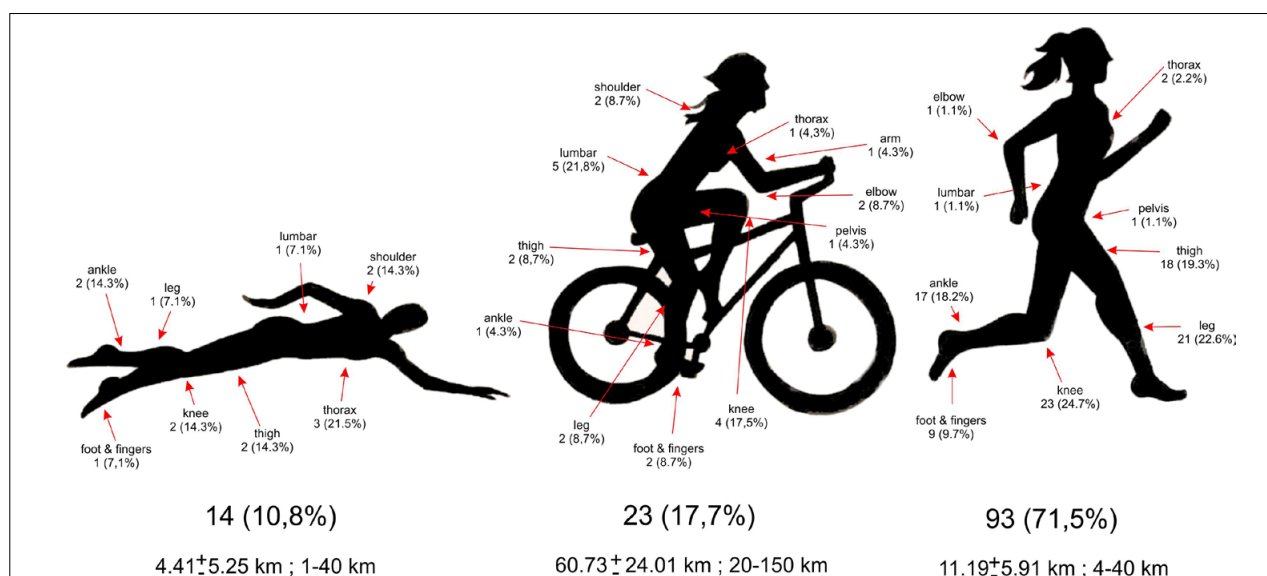
Figure 1 shows the location of injuries distributed by sport modality, as well as the frequency and percentage of injuries that occurred in each modality and the values of the average, standard deviation, minimum, and maximum of the training course of each modality.

Table 1 shows the frequency and percentage of types and the mechanism of injuries distributed by the sports category.

Table 2 shows the relationship, obtained from the application of the binary logistic regression model, between the occurrence of injury over the previous

**TABLE 1.** TYPE AND MECHANISM OF INJURIES

| Variables                       | Sport      |            |            | Total      |
|---------------------------------|------------|------------|------------|------------|
| Type of injury                  | Swim-ming  | Cycling    | Running    |            |
| Fracture                        | 1 (20%)    | 4 (80%)    | 0          | 5 (3.8%)   |
| Muscle contusion/strain/rupture | 6 (14.6%)  | 5 (12.2%)  | 30 (73.2%) | 41 (31.5%) |
| Meniscal injury                 | 0          | 0          | 6 (100%)   | 6 (4.6%)   |
| Luxation                        | 1 (14.3%)  | 1 (14.3%)  | 5 (71.4%)  | 7 (5.4%)   |
| Sprain/Ligament injury          | 3 (15%)    | 1 (5%)     | 16 (80%)   | 20 (15.4%) |
| Tendinopathy                    | 2 (9.5%)   | 2 (9.5%)   | 17 (81%)   | 21 (16.2%) |
| Inflammatory injury             | 1 (4%)     | 6 (24%)    | 18 (72%)   | 25 (19.2%) |
| Low back pain (symptomatology)  | 0          | 3 (75%)    | 1 (25%)    | 4 (3.1%)   |
| Laceration                      | 0          | 1 (100%)   | 0          | 1 (0.8%)   |
| Mechanism of injury             | Swim-ming  | Cycling    | Running    | Total      |
| Excess training                 | 5 (8.9%)   | 8 (14.3%)  | 43 (76.8%) | 56 (43.1%) |
| Temperature                     | 1 (100%)   | 0          | 0          | 1 (0.8%)   |
| Irregular floor                 | 2 (10.5%)  | 1 (5.3%)   | 16 (84.2%) | 19 (14.6%) |
| Bike (equipment)                | -          | 2 (100%)   | -          | 2 (1.5%)   |
| Fall                            | 0          | 5 (50%)    | 5 (50%)    | 10 (7.7%)  |
| Impact with another athlete     | 1 (50%)    | 0          | 1 (50%)    | 2 (1.5%)   |
| Overexertion                    | 1 (6.3%)   | 3 (18.8%)  | 12 (74.9%) | 16 (12.3%) |
| Does not know                   | 4 (16.7%)  | 4 (16.7%)  | 16 (66.6%) | 24 (18.5%) |
| Total                           | 14 (10.8%) | 23 (17.7%) | 93 (71.5%) | 130 (100%) |



**FIGURE 1.**



**TABLE 2.** RELATIONSHIP BETWEEN THE EVENT OF PRESENCE OF INJURY AND VARIABLES ABOUT NON-MODIFIABLE SAMPLE FACTORS AND TRIATHLON PRACTICE CHARACTERISTICS

| Variables  | OddsRatioCrude (CI 95%); p-value |
|--|----------------------------------|
| Gender (female*) male  | 1.2 (0.6-2.4); 0.602             |
| Age group ( $\geq 40$ years old*) until 39 years old             | 1.3 (0.71-2.4); 0.392            |
| Years of practice (until 5 years*) $> 5$ years                   | 1.4 (0.8-2.5); 0.300             |
| Weekly training (until 4 times*) $\geq 5$ times                  | 1.0 (0.4-2.4); 0.964             |
| Duration of training per session (until 2 hours*) $> 2$ hours    | 1.4 (0.7-2.8); 0.407             |
| Coach supervision (no*) yes                                      | 1.7 (0.9-3.5); 0.116             |
| Formats of triathlon competition (Short Distance*) Long Distance | 1.3 (0.7-2.5); 0.471             |

\* Class reference

12-month period and the gender, age group, years of practice, weekly frequency and duration of the training, coach supervision, and formats of triathlon competition. No statistically significant probabilities were obtained in any of the variables analyzed.

## DISCUSSION

The main finding of this study was a higher prevalence of injuries in the triathlete sample analyzed. Attention is drawn to the fact that the sample of this study may involve amateur and professional triathletes since the only criterion for the power of this national competition is to be federated into a triathlon club. When comparing the similar period of data collection, our data of a 12-month period (55%) are close to those obtained by Korkia et al.<sup>6</sup> that showed a prevalence of 47% of injuries in the last year, in 155 British triathletes. The Galera et al.<sup>7</sup> study also revealed a high prevalence (52.4%) in 309 triathletes from a French league in the past season (2007-2008).

Egermann et al.<sup>8</sup> analyzed a lifetime prevalence of 656 triathletes who participated in Ironman Europe 200 and at least one injury was experienced by 74.8% of triathletes. Similar data were obtained by the Bertola et al.<sup>9</sup> study that evaluated 190 triathletes and revealed 76% of injured athletes during training. Our data revealed a value of 69% during the whole triathlon practice.

The data of injury incidence is difficult to compare across studies because of methodology, design, and particularly the injury definition are different. Our results showed an injury rate of 2.39 injuries per 1,000 hours of triathlon training. Korkia et al.<sup>6</sup> revealed a value of 5.4 injuries per 1,000 hours of triathlon

training. Andersen et al.<sup>10</sup> showed an incidence of illness of 5.3/1,000 athlete-days and Zwingerberger et al.<sup>11</sup> an incidence of 0.69 (retrospective) and 1.39 (prospective) per 1,000 training hours.

The most common type of injury observed in our study was muscle contusion/strain/rupture (31.5%), followed by inflammatory injury (19.2%), tendinopathy (16.2%), and sprain/ligament injury (15.4%). Bertola et al.<sup>9</sup> revealed similar data, showing a higher percentage of injuries classified by muscle injuries. The Galera et al.<sup>7</sup> study reported 44.5% of tendinopathies and 35% of muscle injuries. Andersen et al.<sup>10</sup> reported that the most prevalent types of acute injuries were contusions, fractures, and sprains. The classifications of injury types are different between studies, making it difficult to compare them.

Acute injuries are relatively rare in triathlon, and muscle contusion can occur due to a large number of athletes very close to crossing the buoys during the sea race, the falls during cycling, and to mounting and dismounting the bicycle at the start and finish of the cycling leg. Lower limb muscle strains may occur during water or beach exit, or during running in transition area<sup>12</sup>.

Chronic injuries are the most common and may be caused by longer training hours, poor technique or equipment, a lack of running experience, and poor muscle elasticity<sup>5,12</sup>. Besides that, the lack of time to regain the neuromuscular and elastic efficiency indispensable for proper running style can be factors for chronic injuries. The change from concentric to eccentric contractions and the unloaded cycling phase to pass to the transition for the running cycle is a delicate phase that makes athletes more prone to knee pain during the first kilometers of running (the change between muscle activation of concentric contractions in cycling to stretch-shortening in running)<sup>12,13</sup>.

Regarding the site of injury, our data showed a higher prevalence on the knee (22.3%), followed by the leg (18.5%), thigh (16.9%), and ankle (15.4%), i.e., the lower limbs. The lower limbs are requested in the three triathlon modalities and are increasingly required because of the order of the sports categories (swimming, cycling, and running), which may lead more rapidly to high levels of fatigue in these anatomical parts than in others.

Many knee injuries from running are the same as those observed while cycling and include patellofemoral stress syndrome, iliotibial band, and patellar tendinosis<sup>5</sup>. Injuries on the knee may result from impact,

positioning on the bike, and errors in the execution of movements during the race. The cycle-run transition, sudden increases in training distance or intensity, the high impact during the running style, the hill running, and running on steeply cambered roads maybe increase the stress on the iliotibial band (iliotibial band friction syndrome during long-distance running). Besides that, the combination of an anatomical variant of the lower extremities with improper use of a racing bicycle, the use of an inadequate frame, incorrect cleat pedal alignments, the excessive height of the saddle, or too far-back saddles can stress the tensor fasciae latae and iliotibial band muscle<sup>12</sup>.

Lower-leg injury can result from the lower leg's shock-absorbing forces progressing from the foot to the knee. Thigh pain can result from overuse injuries of the hamstrings<sup>5</sup>.

Data from the Galera et al.<sup>7</sup> study revealed that ankle (20.6%), knee (18.3%), and thigh (15%) were the anatomical sites that presented the most injuries. The most prevalent sites of overuse injuries in the Andersen et al.<sup>10</sup> study were the knee (25%) and lower leg (23%). The Korkia et al.<sup>6</sup> study reported that 38% of all injuries affected the ankle/foot, 32% the knee, and 22% the lower leg.

Our study revealed that the main mechanism of injury reported by athletes was excess training (43.1%), and some athletes (14.6%) told that the irregular floor was the factor that caused the injury. The same was observed in the Korkia et al.<sup>6</sup> study that revealed that overuse was the main cause of injury in 41% of cases, and in the Zwingerberger et al.<sup>11</sup> study, overuse was responsible for 29% of injuries.

To reduce the number of overuse injuries, it is important to develop techniques in the three disciplines of triathlon, which includes training at an optimal frequency and load interspersed with an adequate rest phase and correction of biomechanics technique<sup>12</sup>. Besides that, excessive downhill running must be avoided, varying the direction, track, and changing the mechanics of one's running stride. All these considerations may decrease the applied load to the lower extremities<sup>1,12</sup>.

Running was the sports category responsible for the highest percentage of injuries in our study (71.5%), followed by cycling (17.7%) and swimming (10.8%). The same was observed in other studies<sup>6,7,9,11</sup>. The running stage of a triathlon is considered to be the most important leg of the race. Swimming has a significantly lower injury rate than the other two

disciplines in a triathlon, probably because in this triathlete sample the amount of time spent swimming was significantly smaller than the time spent cycling or running, and the triathletes usually devote less time to swimming training than to the other disciplines.

Training errors, anatomical factors, training surfaces, transition, shoes, and training equipment can be responsible for running injuries<sup>12</sup>. The running phase is the category that finishes the triathlon race, when the triathlete has already been subjected to physical and psychological stress and fatigue in the preceding stages<sup>1,13,14</sup>. Further, to start the running race, the triathlete has to prepare their "running legs" for the impact after a period of lower-extremity exertion suspended in water and then fixed on the radius of bicycle pedals<sup>13</sup>.

This study presents some limitations, including the application of a questionnaire based on self-reporting that depended on the participant's memory. The difficulty involved with the self-reporting of injuries by triathletes is acknowledged. The risk of recall bias is high for retrospective information. Besides that, a self-reporting system by lay-persons can lead to misinformation, which makes the reliability of their injury classification questionable. The ideal would be for the injuries to be evaluated by health professionals.

It is suggested that further studies are carried out to verify whether triathletes perform specific exercises to avoid injuries and which training programs are carried out to develop their strength, flexibility, and muscle power valences.

## CONCLUSIONS

Our data demonstrated a high prevalence of injuries in triathletes in this analyzed sample. The most prevalent type of injuries included muscle injuries (contusion/strain/rupture), and the most affected body area were lower limbs (knee, leg and thigh). Overuse was the main mechanism of injuries.

It is important to understand the type, site, and mechanisms of injuries to develop appropriate injury prevention programs.

## Conflict of interest

None

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### Author's Contribution

Beatriz Minghelli: Elaboration of the article; statistical analysis; literature review; article writing; formulation of methodological questions.

Catarian Jesus, Ilvanda Martins and Joana Jesus: Data collection; assistance in statistical analysis, and bibliographic review

## RESUMO

**INTRODUÇÃO:** O triatlo é constituído por três modalidades: natação, ciclismo e corrida. Uma maior frequência de treinamento, fatores ambientais e fatores extrínsecos (técnica e equipamento) podem levar a lesões. O objetivo deste estudo foi determinar a epidemiologia de lesões em triatletas, bem como seu tipo, localização, mecanismo de lesão e fatores de risco.

**MÉTODOS:** A amostra foi constituída por 174 triatletas que participaram do Campeonato Português de Clubes de Triatlo, sendo 131 (75,3%) do sexo masculino, com idades entre 18 e 70 anos ( $36,09 \pm 11,03$ ). O instrumento de medida foi um questionário referente à caracterização da população e aspectos relacionados à modalidade e lesões.

**RESULTADOS:** Cento e vinte (69%) atletas referiram ter tido uma lesão desde que iniciaram a sua prática e 95 (54,6%) sofreram uma lesão no último ano, totalizando 130 lesões. Verificaram-se 2,39 lesões por 1.000 horas de treinamento em triatlo. As lesões mais comuns incluíram contusão muscular (31,5%) e lesões inflamatórias (19,2%), localizadas no joelho (22,3%) e na perna (18,5%). O excesso de treinamento (43,1%) foi o mecanismo que levou à maior ocorrência de lesões. Quatorze (10,8%) lesões ocorreram durante a prática de natação, 23 (17,7%) durante o ciclismo e 93 (71,5%) durante a corrida. Não foi observada significância estatística entre os fatores de risco analisados com a ocorrência de lesões.

**CONCLUSÕES:** A prática de triatlo esteve associada a uma alta prevalência de lesões, sendo a contusão, o joelho e o excesso de treinamento, o tipo, a localização e o mecanismo mais comuns de lesão. É necessário criar estratégias de prevenção de lesões, incluindo treinamento específico e adequação do material utilizado pelo atleta.

**PALAVRAS-CHAVE:** Ciclismo/lesões. Corrida/lesões. Natação/lesões. Epidemiologia. Prevalência. Ferimentos e lesões.

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# Diabetic foot and its serial treatment in high-risk patients: focusing on the individual

 Eli Ávila Souza Júnior<sup>1</sup>  
 Raul Silva Simões de Camargo<sup>2</sup>  
 Tiago Soares Baumfeld<sup>3</sup>  
 Daniel Soares Baumfeld<sup>3</sup>  
 Benjamin Soares Dutra<sup>3</sup>

**1.** Médico Ortopedista – Universidade Federal de Alfenas – Unifal, Alfenas, MG, Brasil.  
**2.** Acadêmico de Medicina – Universidade Federal de Minas Gerais – UFMG, Belo Horizonte, MG, Brasil.  
**3.** Médico Ortopedista – Hospital Felício Rocho, Belo Horizonte, MG, Brasil.

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## SUMMARY

**OBJECTIVES:** To assess knowledge about diabetic foot, care measures, and the importance attached to serial treatment in a group of high-risk diabetic foot patients.

**METHODS:** This is a cross-sectional study, carried out in a tertiary hospital, with 25 patients undergoing serial treatment for diabetic foot. The tabulation of the data occurred through the use of three methodological figures: core idea, key expressions, and the collective subject discourse.

**RESULTS:** It became evident that even among high-risk patients with diabetic foot, there is no complete knowledge about the definition of the disease. Despite this, all participants reported practicing daily care measures, including frequent inspection of the feet, food care, and attention to footwear. Regarding the importance of serial treatment, there was unanimous recognition of the relevance of this practice, which improves self-care discipline, optimizes the understanding of the disease, and helps to prevent progression.

**CONCLUSIONS:** Authentic speeches in the context of a pathology of considerable prevalence manifested, in an unprecedented way, with conceptions about its definition, care measures, and importance of serial treatment in a high-risk group.

**KEYWORDS:** Foot diseases. Diabetic foot. Amputation. Bioethics.

## INTRODUCTION

Diabetic foot is one of the most devastating chronic complications of diabetes mellitus due to the large number of cases that evolve to amputation<sup>1</sup>. For the individual, it brings repercussions in their personal life, affecting their self-image, self-esteem, and role in their family and in society, and, if there are physical limitations, it can cause social isolation and depression<sup>2,3</sup>.

The World Health Organization<sup>4</sup> defines therapeutic education as the training of patients and family members in the skills for the management of treatment or for special adjustments and for the prevention of complications from the disease. Its importance is recognized for the treatment of chronic diseases, such as diabetic foot, in which the patient is responsible for long-term care in order to prevent complications<sup>5</sup>.

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CORRESPONDING AUTHOR: Eli Ávila Souza Júnior

Alameda Libânio, 72, Condomínio Jardim da Colina, Alfenas, MG, Brasil – 37130-000

E-mail: elijr42@yahoo.com.br

The results of clinical trials assessing the preventive efficacy of training diabetic patients for preventing diabetic foot lesions are not conclusive<sup>6</sup>. The failures seem to be related to the non-adherence of patients to the recommendations received<sup>7</sup>. The issue of care of the feet, from the perspective of patients, a thorough knowledge of the meaning of the disease, of care, its needs, their sufferings and anxieties can point out new directions for therapeutic education so as to improve its results.

For Minayo et al.<sup>8</sup>, qualitative research answers specific questions, considering as the object of study people belonging to a group and with a certain social condition, with a set of meanings, values, beliefs and attitudes. Exploratory research is carried out in areas in which there is little accumulated and systematized knowledge, constituting, in the first step, of a broader investigation, developed when a topic is little explored. Due to its survey nature, it does not contemplate hypotheses that may arise during or at the end of the research.

## METHODS

This is an exploratory-descriptive, cross-sectional study with a qualitative approach, approved by the Research Ethics Committee of the institution.

We elected as inclusion criteria: patients with diabetic foot, a history of any previous amputation caused by diabetes (high-risk patients), undergoing serial treatment in the orthopedic center of the institution. We understood by serial treatment of diabetic foot monthly evaluations in a specialized outpatient clinic, during which the feet are inspected by a specialist who performs several procedures, such as: debridements, special dressings, and providing care instructions for feet at risk.

The study, conducted from 1 April 2019 to February 1 2020 included 25 patients undergoing serial follow-up.

To know and describe the considerations on serial treatment for diabetic foot, under the framework of Social Representations (SR), and the Collective Subject Discourse (CSD) method was chosen since it allows an approximation to the phenomenon under study. CDS consists of a set of procedures for the tabulation of discursive data used to obtain an understanding of a given topic. The analytical procedure was operationalized with the following steps: the selection of key expressions of each discourse,

analogous to the vital tone; identification of the core idea of each key expression, building the synthesis of the content; identification of similar or complementary core ideas; the gathering of key expressions referring to the core ideas<sup>9</sup>.

We then carried out the individual interviews, using three structured questions that addressed: the concept for diabetic foot, the daily practices of care for diabetic foot, and the importance attributed to the serial treatment of diabetic foot. In addition, we characterized the sample, based on gender, age, type of diabetes (I or II), time of diabetes diagnosis, and history of amputation. Previously, the informed consent form (ICF) was signed, per Resolution no. 466/2012 of the National Council of Health.

For the data analysis, the order of the steps was strictly followed.

In the first one, in order to obtain a complete knowledge of the narratives/discourses transcribed, we conducted an exhaustive reading to build an overview and a better understanding of the discursive manifestations.

In the second one, we conducted a reading of each transcript alone, based on each of the questions in the guide.

In the third, after reading the full content of all answers inherent to each one of the three questions from each respondent, we used the instruments from discourse analysis, representing the key expressions (KEs), which are excerpts from the discourse that reveal the essence of the statement, which are found in italics or underlined. In possession of the KEs and after reading each one, we identified the core ideas (CI), which describe in the most concise and accurate way possible the meaning of each one of the discourses analyzed and of each homogenous set of KEs, which will later make up the CSD. In addition to the CI, the KEs can also refer to a methodological figure. This same procedure was carried out with all three questions.

In the fourth step, the Discourse Analysis Instrument was prepared, which accounted for, separately, each CI with their respective similar or complementary KEs.

In the fifth step, we built the collective discourse for each grouping. It was necessary to sequence the KEs of each group, organizing them into a beginning, middle and end. We adopted grammatical connectives to connect the KEs, maintaining the cohesion of discourse.



## RESULTS

The characterization of the sample is detailed in the table below:

**TABLE 1.** CHARACTERIZATION OF THE SAMPLE

|                            |  |
|----------------------------|--|
| Gender                     | Male = 88%<br>Female = 12%   |
| Age                        | Maximum = 91 years<br>Minimum = 39 years<br>Mean = 72 years              |
| Type of diabetes (I or II) | I = 8%<br>II = 92%   |
| Time of diagnosis          | Maximum = 51 years<br>Minimum = 2 years<br>Mean = 18 years               |
| History of amputation      | Toe = 44%<br>Radial = 16%<br>Transmetatarsal = 16%<br>Transfemoral = 20% |

Table 2 demonstrates the core ideas and prevalences found for the three approaches.

**TABLE 2.** CORE IDEAS AND PREVALENCES

| Questions   | Core idea                              | Prevalence |
|---|--|------------|
| In your opinion, what is the concept of diabetic foot?  | Vulnerability to injury and amputation | 40%        |
|   | Doesn't know                           | 30%        |
|   | Manifestation of glycemic disorder     | 20%        |
|   | Consequences of vascular insufficiency | 10%        |
| Do you practice care for the diabetic foot on a daily basis? Which ones?                          | Attention to footwear                  | 50%        |
|   | Dietary care                           | 20%        |
|   | Frequent inspection                    | 30%        |
| In your opinion, what is the importance of the serial treatment for diabetic foot practiced here? | Prevention of progression              | 40%        |
|   | Discipline in care                     | 20%        |
|   | Better understanding of the disease    | 40%        |

The core ideas (CI), keywords and collective subject discourse for the first approach (In your opinion, what is the concept of diabetic foot?) are listed below:

**CI:** Vulnerability to injury and amputation - 40%

**CSD:** "Situation in which any '*small blister*' becomes a *wound*; the *wounds* evolve and quickly lead to *amputation*; the *wounds* under the foot are the ones you have to look out for; well, based on the *amputation* I had on my foot, it is a condition that causes *amputations*"

**CI:** I do not know - 30%

**CSD:** "I understand almost *nothing*, I take care, but I *cannot say*; I *don't know anything* about this issue; honestly, I *do not know anything*"

**CI:** Manifestation of glycemic disorder - 20%

**CSD:** "It is due to *sugar*; a *sugar disorder* in the blood causes this disease on the foot; it is related to the fact that my *glucose is a bit high*; the problem is the *glucose*, it is always "peaking"; *glucose* peaks lead to these complications"

**CI:** Consequences of vascular insufficiency -10%

**CSD:** "It is a *deficiency of vascularization*; it is a *circulatory complication* and from it comes *gangrene*; I think that the *circulation is compromised*, the *blood* is not flowing enough to the foot"

The core ideas (CI), keywords and collective subject discourse (CSD) for the second approach (Do you practice care for the diabetic foot on a daily basis? Which ones?) are listed below:

**CI:** Attention to footwear - 50%

**CSD:** "I'm *never barefoot* and always dry my feet to prevent injuries; I am *cautious with footwear* not to hit it; I wear *footwear* in the countryside; I wear custom *footwear*; I always wear *boots*, I never walk *barefoot*"

**CI:** Dietary care - 20%

**CSD:** "I *do not eat sweets*, only the ones for diabetics; *balanced diet*, my food is usually different from others; I *don't eat sugar*; *controlled diet*; I try to *eat* more natural products"

**CI:** Frequent Inspection - 30%

**CSD:** "I *keep watch of my foot 24 hours* per day; I always *look in the mirror*; I wear light-colored socks to *notice* any bleeding; I see a podiatrist to get my nails cut; I am always *watching* my toe"

The core ideas (CI), keywords and collective subject discourse (CSD) for the third approach (In your opinion, what is the importance of serial treatment for diabetic foot?) are listed below:

**CI:** Prevent progression - 40%

**CSD:** "Monitoring is essential to help in *healing*; I feel that in two months it will *have healed*; I like to carry out the control to *prevent progression*; I think that it is essential to take care for it not to *evolve further*"

**CI:** Discipline in healthcare - 20%

**CSD:** "Diabetic individuals are liars, always making



up excuses, but now *things took a turn* for the better, now I am *more disciplined*; now I *value it more*; I *pay more attention*"

**CI:** Better understanding - 40%

**CSD:** "*I got instruction, it enables me to know how to take care; brings more tranquility to us; you have much more control of what is happening with yourself, control of the situation; it helped me understand a bit better*"

## DISCUSSION

In the sample characterization, we observed that most people with high-risk diabetic foot were elderly (mean age 72 years). A survey conducted in the city of Planura, Minas Gerais, Brasil, also found that the elderly makeup most of the people with diabetic foot who attended a particular health institution<sup>10</sup>. A study performed in Ribeirão Preto, SP, presented a frequency slightly higher for males regarding diabetic patients with foot ulcers<sup>11</sup>. In that study, in which were considered patients previously amputated, the male sample was significantly greater (88%). This finding contrasts with the study conducted at the University of the State of Pará, in which women with diabetic foot were greater in number<sup>12</sup>.

Diabetic foot is a term used to designate the various ailments which may evolve to amputation<sup>13</sup>. In this study, when we investigated the concept of diabetic foot among participants, no complete definition was observed; however, most correlated the disease with some of its characteristics, through core ideas, such as: vulnerability to injury and amputations, manifestation of glycemic disorder, and repercussions of vascular insufficiency. However, 30% could not define anything concerning the pathology. This finding is alarming, considering these are high-risk patients already submitted to previous amputations. It is estimated that 30% to 50% of those who underwent an amputation will require additional amputations within one to three years, and 50% will die within five years from the first amputation of greater level<sup>14</sup>.

The adhesion to examining shoes before putting them on often masks the real information that, in truth, these people were open shoes, therefore, there is no need to look inside them, data that contradicts the literature recommendations, endorsed by the SBD<sup>15</sup> and ADA<sup>16</sup>. Research has shown that, out of 22 diabetic patients participating in a study, 81.8% worn

open shoes<sup>17</sup>. In a city in the interior of Minas Gerais, a percentage of 92% of diabetics wearing inadequate footwear was identified<sup>18</sup>. Among the care measures practiced by the interviewees in this study, the majority (50%) mentioned care with the shoes worn.

Poor glycemic control facilitates the onset and development of chronic complications and increases the risk of neuropathy; however, there are no studies demonstrating a direct relationship between hyperglycemia and amputations<sup>19</sup>. Knowledge about glycemic optimization was also seen in the participants of this study (20%), who mentioned dietary attention as a way to care for diabetic foot.

Acknowledging the value of shared responsibility and of the need for the developing autonomy and participation of diabetic individuals has the potential to improve care because of the likely positive effect of their own satisfaction in the adherence to treatment. On the other hand, patients who do not adhere to treatment have a 50-times-higher probability of having foot ulcerations and are 20 times more likely to be amputated than those who follow the guidelines correctly<sup>20</sup>. This shared responsibility was inferred by 20% of the participants, who recognized the importance of serial treatment for diabetic foot as a way of being more disciplined.

According to the International Consensus on Diabetic Foot, people should undergo, at least, one annual examination of their feet<sup>21</sup>. For those at high risk (including those that have already been submitted to amputation), the examination should be done every one to three months, and in special conditions, even weekly. It ensures that this exam is the essential component for the proper management of this complication, upon an investigation of the protective sensation of the foot, its structure, biomechanics, circulation and skin integrity, through simple and low-cost tests. Forty percent of the respondents acknowledged that serial control is an important way of preventing the progression of the disease.

In the studies by Santos et al.<sup>22</sup>, most individuals who underwent amputations had poor metabolic control, had no access to information on preventive care, did not adhere to the clinical treatment, and had financial difficulties. In addition, amputation and limb loss have a greater impact than any other complication from diabetes, since, in addition to the loss of mobility and independence, anxiety and depression are frequent. The care for diabetic foot improves with a clearer understanding of the factors

that lead to limb loss and a growing consensus on the various measures that must be taken regarding the foot. Forty percent of the participants of this study valued the serial treatment as a way of obtaining more knowledge about the preventive measures and practices for diabetic foot.

## CONCLUSION

It was evidenced that, from a qualitative approach, that even among patients with a high risk for diabetic foot, there is no full knowledge about the definition of the disease; some expressed some understanding, while 30% reported having no knowledge. Despite this, all participants reported practicing daily care, including the frequent inspection of the foot, dietary care, and attention to footwear. Regarding the importance of the serial treatment, the recognition of the importance of this practice was unanimous, with statements that it improves the discipline of self-care, enhances

the understanding of the disease, and helps prevent its progression.

## Ethical Aspects

The authors declare there are no conflicts of interest.

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## Author's Contribution

Eli Ávila Souza Júnior conceived and planned the activities that led to the study and drafting of the paper, in addition to the process of final review; Raul Silva Simões de Camargo and Tiago Soares Baumfeld interpreted the results of the study and participated in the process of the final review; Daniel Soares Baumfeld and Benjamin Soares Dutra participated in the process of final review and approved the final version.

## RESUMO

**OBJETIVOS:** Avaliar o conhecimento sobre o pé diabético, medidas de cuidado e importância atribuída ao tratamento seriado em um grupo de alto de risco de portadores de pé diabético.

**MÉTODOS:** Este é um estudo transversal, realizado em um hospital terciário, com 25 pacientes submetidos ao tratamento seriado do pé diabético. A tabulação dos dados ocorreu por meio da utilização de três figuras metodológicas: ideia central, expressões-chave e o discurso sujeito coletivo.

**RESULTADOS:** Evidenciou-se que mesmo entre pacientes de alto risco do pé diabético, não há conhecimento pleno sobre a definição da doença. Apesar disso, todos os participantes relataram praticar medidas diárias de cuidado, incluindo inspeção frequente dos pés, cuidado alimentar e atenção aos calçados. Sobre a importância do tratamento seriado, foi unânime o reconhecimento da relevância dessa prática, a qual melhora a disciplina dos autocuidados, otimiza a compreensão da doença e ajuda a prevenir a progressão.

**CONCLUSÕES:** Discursos autênticos no âmbito de uma patologia de considerável prevalência manifestaram, de forma inédita, as concepções sobre definição, medidas de cuidado e importância do tratamento seriado em um grupo de alto risco.

**PALAVRAS-CHAVE:** Doenças do pé. Pé diabético. Amputação. Bioética.

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# Preparing a high-performance surgical team: lessons from 11,000 surgeries

 Andre Roncon Dias<sup>1</sup>

<sup>1</sup>. Instituto do Câncer, Universidade de São Paulo, São Paulo, SP, Brasil.

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## SUMMARY

**INTRODUCTION:** *In the future, surgery will be centralized in hospitals with the best value (excellent results with reduced cost). High-performance teams will be required; however, available data concerning the specific abilities necessary to build and manage them are poor.*

**OBJECTIVES:** *Share the set of competencies and skills established to build and lead a high-performance general surgery team.*

**METHODS:** *In November 2012, a general surgery team started its activities at a tertiary hospital in São Paulo, Brazil. The model consisted of a high volume performed by a small team. Experienced surgeons, motivated, and with technical and moral excellence were selected. A sense of unity was created and goals were shared. Complex cases were discussed daily and a prospective database to follow outcomes was established. The payment value was above the market.*

**RESULTS:** *In 6 years and 4 months, 11,006 surgical procedures were performed (8,597 electives and 2,409 in an urgent setting). Cholecystectomy was the most common procedure (4,101, being 3,676 electives), followed by inguinal hernioplasty (n: 1,827) and appendectomy (n: 925). A total of 449 elective oncologic procedures were performed. The surgical site infection rate in clean procedures was 0.12%, 80 patients required re-do surgery (2.4% in an urgent setting and 0.2% of the electives). There were 22 postoperative deaths (0.66% for urgent and 0.07% for electives), 5 of the 6 deaths in elective patients were in oncologic cases.*

**CONCLUSION:** *A competent surgical team, inserted in a model that favors performance and values the individual can deliver high volume with exceptional results.*

**KEYWORDS:** Patient Care Team. General surgery. Health Management. Outcome Assessment, Health Care.

## INTRODUCTION

Healthcare expenditure is a major issue worldwide. As life expectancy increases and new exams, treatments, and procedures appear, income destined for healthcare rises. Healthcare is a right, and there is a moral obligation to provide it; nevertheless, there are income restrictions and even more complex decisions, such as where to place a limited budget. In this context, attention should be given to value in healthcare. Let us consider the value of

a determined treatment as the result achieved per monetary cost<sup>1-3</sup>.

Great value can be achieved by a high-performance team, who is able to perform high volume at high speed, delivering excellent results, ultimately resulting in low monetary cost. But, how to build and lead this team? In sports and business, literature is vast; however, available data about high-performance medical teams is scarce<sup>4</sup>.

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CORRESPONDING AUTHOR: Andre Roncon Dias

Rua Pedroso Alvarenga, 1208, 11º andar, Itaim Bibi, SP, Brasil – 04101-100

E-mail: roncon86@hotmail.com

## OBJECTIVES

The main objective of this article is to present the building and management of a high-performance general surgery team, sharing the ideas and solutions implemented and the results achieved.

## METHODS

In 2012, a tertiary hospital in Sao Paulo, Brasil (Hospital Santa Cecilia) required a high-volume general surgery team. The hospital belonged to a health-care insurance company that requested volume under a low budget.

### Volume

The insurer needed a general surgery group to perform monthly up to 140 surgical procedures and 1,200 clinical evaluations (Table 1). The team would be responsible for inpatients and available 24/7 for evaluations of patients from other medical specialties, including the emergency department (ED) and intensive care unit (ICU). The ED received 4,500 to 6,000 visits monthly, and the ICU had 20 beds.

**TABLE 1.** HOSPITAL'S VOLUME REQUIREMENTS

| Surgical procedures |                   |       |
|---------------------|-------------------|-------|
|                     | Electives         | 100   |
|                     | Urgency/emergency | 30-40 |
| Evaluations         |                   |       |
|                     | Inpatients        | 400   |
|                     | Outpatients       | 800   |

### Team structure

The team was structured on weekdays with one surgeon in the outpatient clinic (30 patients in 6h period) and two surgeons and one scrub nurse in the hospital. On weekends, one surgeon was responsible for the inpatients and the second stayed on call at home.

The 2 surgeons in the hospital were responsible for the team's inpatients, evaluations requested from other specialties, elective surgical procedures scheduled, and any eventual urgent surgical pathology. After the end of their activities, both remained available at home (one was responsible for all calls and in-hospital evaluations, and the other available for surgery when necessary). Surgeries started at 8 am, and two surgical rooms were available for the team daily.

The outpatient clinic opened in September 2012, and the hospital's activities were started in the following month.

Initially, 4 electives were programmed every day, this was raised to 5 as demand expanded. After June 2016, another team of 2 surgeons and 1 scrub nurse were assigned for oncologic procedures, with one day per week for surgery and one period for outpatients (20 patients in 4h).

Initially, the whole team consisted of four surgeons and one scrub nurse. After a few months, a couple of surgeons were incorporated and, in June 2018, five more surgeons and one scrub nurse were added.

### Team selection

Once the structure was defined, surgeons were interviewed. The objective was to identify if they were fit for the intended model. The profile desired included technical competency, ability to work in a group, commitment to the proposed goals, and plasticity to share the team mindset. The main questions during this process were: résumé and medical formation discussion, experience in general and laparoscopic surgery, previous jobs, ethics, ability to work under stress. They were also informed on the volume and results desired, and the monetary value.

Surgeons were required to be board certified in general surgery and proficient in laparoscopic access.

The scrub nurse underwent a similar interview and was also board certified.

### Patient management

The team had access to a cell phone app where all inpatients were presented daily. Complex outpatient cases were also discussed. Decisions were shared and the leader moderated the debates and was ultimately responsible for hard decisions.

Protocols were created and implemented to ensure patient safety. Flowcharts contemplating the clinical thinking and exams recommended for the most common surgical pathologies observed in the ED were also developed. Pre-operative protocols were created for outpatients.

A multidisciplinary culture focused on the patient was established in the hospital, engaging anesthesiologists, nurses, and other teams in achieving high-performance.

The surgical ward required profound culture and equipment changes to support the volume desired without sacrificing safety. Standardization

of the equipment used in the most common procedures was implemented, allowing easy and efficient stock management.

### Payment

Patients were treated in facilities from their insurance company, making no direct payment to the surgical team. The monetary value was previously agreed on and was based on the number of hours the team worked. The value for staying on call at a distance was established as 1/3 of the one in-hospital. The capital per hour worked was above the market.

## RESULTS

In 76 months (November 2012 to February 2019), 11,006 surgical procedures were performed (Table 2). A total of 8,597 were elective and 2,409 were performed in a urgency/emergency setting. Women accounted for 52% ( $n$ : 5,762) of the patients. The most common elective procedures were cholecystectomy ( $n$ : 3,676) and inguinal hernia repair ( $n$ : 1,827), while appendectomy was the most frequent urgent surgery ( $n$ : 925). The mean hospital stay was 2.1 days. The suspension index was 0.1%, with upper respiratory tract infection as the most common cause. Surgical site infection in clean elective surgery was 0.12% and higher in incisional hernia repair (3.3%).

Eighty patients required surgical redo, 2.4% in urgency patients and 0.2% in elective ones. In the period, 22 (0.19%) deaths occurred: 0.66% and 0.07% for urgency and elective, respectively. Thirteen deaths were of oncologic patients, including 5 out of the 6 deaths that occurred in elective patients. Deaths not related to the surgical procedure (e.g., terminal or palliative patients who required a feeding tube and

eventually died due to clinical complications unrelated to the surgical procedure) were not computed.

Surgery volume increased over the years; at the beginning, the number of elective cases was restricted, slowly rising over time. Urgent procedures also increased, except in 2017 (Table 2). A boost in volume occurred in 2018 after expanding the team.

Considering the most performed surgery, i.e., cholecystectomy ( $n$ : 4,121), laparoscopic access was performed in 4,101 cases with conversion to open surgery in 0.2% of them. Five reoperations were necessary (0.02%), three (0.07%) were due to biliary leakage, and two (0.04%) due to iatrogenic intestinal perforation. There was one death, an 84-year-old female admitted in the ED in septic shock due to acute cholecystitis (0.2% mortality, considering the 445 urgent cholecystectomies performed).

In total, 449 oncologic procedures were performed, with a mortality rate of 1.1%. Most procedures were performed laparoscopically (67%). Among the major procedures performed, there were: low abdominal resection ( $n$ : 61), gastrectomy ( $n$ : 52), distal pancreatectomy ( $n$ : 19), pancreaticoduodenectomy ( $n$ : 17), hepatectomy ( $n$ : 10), esophagectomy ( $n$ : 6). No reoperations or mortality were observed in pancreaticoduodenectomy or esophagectomy. Positive margins occurred in 10% of the pancreaticoduodenectomies.

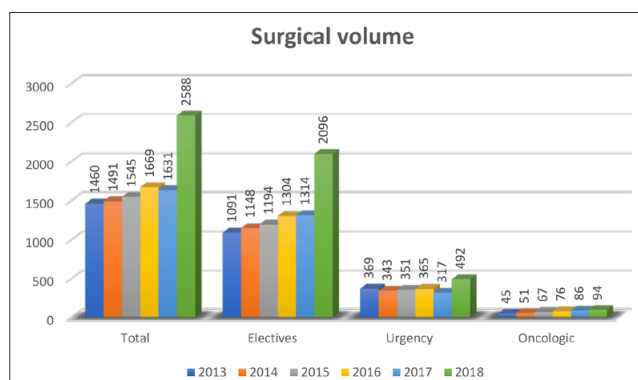
Team member's suggestions and demands were always considered and incorporated whenever possible. Individual feedback was given monthly, and when a negative event or criticism occurred, immediate feed-back was given and a meeting arranged for the same week. Highly demanding patients or families (for medical or social condition) were followed-up closely by the team leader (daily if necessary). In 76 months, only 1 surgeon was replaced. Periodic social meetings were promoted and an official meeting occurred at the end of every year; the team's performance and results were presented, followed by a social gathering along with their respective families.

Rare procedures were performed during the 72 months period, some were presented and received honorary mentions at the Brazilian Digestive Week, others were published<sup>5-7</sup>.

## DISCUSSION

Healthcare in Brasil is complex. Although there is a universal healthcare system (SUS) run by the government and sustained with taxes, private health

**TABLE 2.** SURGICAL PROCEDURES PER YEAR.



\* 2012 and 2019 not contemplated



insurances are common. This happens because SUS has few isles of excellence and long waiting lists for medical assistance and exams.

There are private insurances for all budgets and the quality of healthcare granted scales accordingly. More recently, few insurance companies started building their own healthcare system. Instead of reimbursing one-third of expenses, everything, from exams to surgeries, is done in their units. By doing so, their expenditure is limited and the profits potentialized. This allows them to provide budget health insurance to industries and companies with thousands of low-income employees.

However, the best interest of patients, healthcare providers, and the insurer may not always be the same.

In this context, one major insurer asked for a budget high-volume general surgery team. The main issue was remaining inside their stipulated budget. The proposal was to deliver good value per treatment. This would be achieved by having a high-performance team, in which members could deliver a big volume with great quality (low complication rates). As the budget was limited, having fewer members allowed greater individual payment (with an above-market capital per hour worked). Additionally, achieving results of excellence ultimately reduced the value of any given treatment, indirectly saving money.

Let us consider the most common procedure performed, the cholecystectomy. This is considered a simple and low-risk surgery; however, its complications may cost up to 10,000 USD (300,000 USD during the patient's life)<sup>8</sup>. Comparing the results of our 4,121 cases with two huge series ( $n$ : 39,238<sup>9</sup> e 9,542<sup>10</sup>), our major complications index was inferior (0.07% vs 1%), as was iatrogenic biliary tree lesions (0.07% vs 0.17-0.63%) and re-dos (0.02% vs 0.9%). Safety was always a concern and peritoneum was created in open fashion, reducing the risk for iatrogenic bowel injury (incidence of 0.04%, compared to 0.06%<sup>9</sup>).

Indeed, the results were better than anticipated. The hospital infection rate was very low. This may be partially credited to the safety measures established. Patients were educated, took a chlorhexidine bath before surgery, trichotomy was performed in the operating room, prophylactic antibiotics were used, among other measures. Incisional hernias had the greatest infection index, but still very low compared to the literature (3.3% vs up to 16%)<sup>11</sup>. For instance,

those with body mass index  $>30$  were lectured and underwent endocrine and nutritional support, losing weight before surgery.

It is a strong belief that the main action to achieve good results was the team's selection. They were required to have technical and moral excellence. Experience, knowledge, no previous ethical faults, capacity to work in a group, resilient personality, and traits of perseverance and self-control were must-have virtues. The interviewing process and curriculum analysis helped to identify the correct professionals, and the solid project with good payment attracted them. To keep these professionals, a sense of unity was created. They were part of a uniform team, with shared mindset and objectives; all were important, empowered, had their opinions valued, and could share responsibility.

Before starting, few meetings were arranged, so people got acquainted, questions were answered, problems and possible solutions anticipated, and the objectives were clearly established. During the years, this was nurtured. To help build a strong uniform team, periodic meetings were promoted.

Another important issue was the progressive increase in workload. This allowed the team to get familiar with the system, the hospital and other teams, and to identify and solve issues that restricted productivity. With time, everybody's (surgical team, hospital staff) capacity to tolerate a higher volume with the same, or even lower index of flaws, increased.

Since the beginning, complex procedures were centralized in determined surgeons, while most could be scheduled on any given day. Laparoscopic gastroesophageal reflux for instance had unsatisfactory results initially. After identifying this, the procedure was restricted, those willing to learn were invited to observe first, then perform under supervision, and only then allowed to operate without an observer. Superior results were then achieved.

As exemplified above, periodic checks were necessary to verify results and eventually provide corrections. Besides the insurer quality metrics (i.e.: median length of hospital stay, surgery cancellation, ICU stay, treatment value, patient complaints and compliments), a prospective database was created and fed daily. Both parts shared and discussed these indexes regularly, collaborating with ideas and measures to keep improving value.

In a world of increasing healthcare costs, focus should be given to value. In general surgery, a

high-performance team is a cost-effective option. Although literature concerning building and managing such medical teams is scarce, there are probably countless ideas and methods worth considering. The ones presented here are based on the achieved results

and intended to broaden thinking and help leaders make better decisions.

### Author's Contribution

All authors have contributed equally to this work

## RESUMO

**INTRODUÇÃO:** No futuro as cirurgias serão centralizadas em hospitais que tenham melhor valor (resultados excelentes com custo reduzido). Equipes de alta performance serão necessárias, entretanto a literatura acerca das habilidades específicas para montar e gerir essas equipes é muito pobre.

**OBJETIVOS:** Compartilhar as competências e habilidades estabelecidas para construir e liderar uma equipe de cirurgia geral de alto desempenho.

**MÉTODOS:** Em novembro de 2012, a equipe de cirurgia geral iniciou suas atividades em um hospital terciário na cidade de São Paulo (SP, Brasil). O modelo consistia em produzir alto volume com um time pequeno. Cirurgiões experientes, motivados e com excelência técnica e moral foram selecionados. Um senso de unidade foi criado e objetivos coletivos estabelecidos e compartilhados. O pagamento estava acima da média do mercado.

**RESULTADOS:** Em 6 anos e 4 meses, 11.006 procedimentos cirúrgicos foram realizados (8.597 eletivos e 2.409 de urgência/emergência). A colecistectomia foi o procedimento mais comum (n: 4.101, sendo 3.676 eletivas), seguida da hernioplastia inguinal (n: 1.827) e da apendicectomia (n: 925). Um total de 449 cirurgias oncológicas eletivas foram realizadas. A infecção de sítio cirúrgico em procedimentos limpos foi de 0,12% (2,4% para cirurgias de urgência e 0,2% nas eletivas). Ocorreram 22 óbitos cirúrgicos (0,66% na urgência e 0,07% nas eletivas), 5 dos 6 óbitos eletivos ocorreram em pacientes oncológicos.

**CONCLUSÃO:** Uma equipe competente, inserida em um modelo que favorece o desempenho e valoriza o indivíduo é capaz de grande volume com resultados excepcionais.

**PALAVRAS-CHAVE:** Equipe de Assistência ao Paciente. Cirurgia geral. Gestão em Saúde. Avaliação de Resultados em Cuidados de Saúde.

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# Knowledge and attitudes towards COVID-19 among emergency medical service workers

 Aslı Vatan<sup>1</sup>  
 Ertuğrul Güçlü<sup>1</sup>  
 Aziz Öğütlü<sup>1</sup>  
 Fulya Aktan Kibar<sup>2</sup>  
 Oğuz Karabay<sup>1</sup>

1. Department of Infectious Diseases and Clinical Microbiology, Sakarya University Training and Research Hospital, Sakarya, Turkey.  
2. Department of Public Health, Sakarya University Training and Research Hospital, Sakarya, Turkey.

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## SUMMARY

**OBJECTIVE:** Good knowledge of the coronavirus disease 2019 (COVID-19) among healthcare workers is essential for keeping health systems active and controlling the outbreak. We aimed to investigate the knowledge and attitudes of Emergency Medical Service (EMS) employees who fight COVID-19 at the forefront.

**METHODS:** A total of 400 EMS workers (doctors, nurses, emergency medical technicians, paramedics, and ambulance drivers) were included in this study. Knowledge, attitude, and preventive behaviors for COVID-19 were evaluated using an online questionnaire.

**RESULTS:** A total of 275 EMS workers participated in the study with a response rate of 68.8%. The respondents reported that their highest common sources of knowledge about COVID-19 were social media and television (n=240, 88%). Overall, > 96% of the participants had adequate knowledge about the transmission routes of COVID-19. Among the respondents, 36% of them were unaware of the correct hand washing or scrubbing technique. In addition, 78% of the participants had poor knowledge about floor and surface disinfection. The majority of the participants exhibited inaccurate attitudes toward the use of personal preventive equipment. More than half of EMS workers (52%) agreed that a surgical mask is not enough during the procedures that do not generate aerosol. Moreover, a significant proportion of the participants (66%) perceived that a N95 mask is required.

**CONCLUSIONS:** As a consequence, although emergency workers have sufficient basic knowledge about COVID-19, there is a need for postgraduate training in many subjects.

**KEYWORDS:** coronavirus disease 2019, emergency medical service, knowledge and attitudes, questionnaire

## INTRODUCTION

COVID-19 initially manifested as a cluster of pneumonia cases of unknown etiology in Wuhan, Hubei Province of China on 31 December 2019<sup>1</sup>. The causative agent was identified as a novel coronavirus that had not been previously seen in humans<sup>2</sup>. The World Health Organization (WHO) determined a new name

for the epidemic disease caused by this virus – coronavirus disease 2019 (COVID-19) and on January 30<sup>th</sup>, declared the coronavirus outbreak a Public Health Emergency of International Concern<sup>3</sup>.

According to the current evidence, the main routes of transmission are human-to-human via respiratory

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CORRESPONDING AUTHOR: Aslı Vatan  
Sakarya University Training and Research Hospital, Adnan Menderes Street, 54000, Sakarya, Turkey  
Tel: +90 530 2889936 / Fax: +90 264 2759192  
E-mail: asli\_oner@hotmail.com

droplets, direct contact, or fomites<sup>4</sup>. The common clinical manifestations of the disease are fever, dry cough, shortness of breath and bilateral patchy infiltrates on imaging<sup>4</sup>. The fatality rate of the COVID-19 is currently reported as approximately 4%, which is much lower than that of the Severe Acute Respiratory Syndrome (SARS) and Middle East Respiratory Syndrome (MERS)<sup>5</sup>.

Healthcare professionals who are at the front line of the COVID-19 outbreak fight are at high risk of contracting the infection<sup>6</sup>. In a study from China, the rate of transmission to health professionals found was 29%<sup>7</sup>. Data from China's National Health Commission showed that more than 3,300 healthcare workers have been infected as of early March. In Italy, 20% of responding healthcare workers were infected, and some have died<sup>8</sup>.

Emergency medical service (EMS) workers are first responders who anticipate close contact with affected persons, thus, EMS workers are expected to be at high risk of COVID-19 infection. It is very important for the EMS staff to have knowledge about this disease as they need to respond quickly and closely to COVID-19 patients in a narrow area within the ambulance. However, unlike managing a patient in the controlled environment of hospitals, care and transport by EMS present distinct challenges because of the nature of the setting, enclosed area during transport, frequent need for rapid medical response, interventions with limited medical information. Therefore, insufficient knowledge and awareness regarding COVID-19 in the field of EMS is associated with an increased risk of contracting the infection. In addition, this situation can negatively affect the level of anxiety of EMS personnel and the appropriateness of their medical decisions<sup>9</sup>.

In this study, we aimed to investigate the knowledge and attitudes of EMS workers about COVID-19.

## METHODS

### Participants

In total, 400 EMS workers (doctors, nurses, emergency medical technicians, paramedics, and ambulance drivers) were employed in Sakarya province. An online questionnaire was sent to EMS workers via their e-mail accounts. The study was performed per the ethical considerations of the Helsinki Declarations. The Ethics Committee of the Sakarya University School of Medicine approved this study.

## Questionnaire

The study questionnaire was administered 2 weeks after the start of the COVID-19 outbreak, between 1-15 April in Turkey. The survey questions were designed based on the recommendations of the World Health Organization (WHO) and modified from previously published research articles<sup>10,11</sup>. The survey consisted of two sections with 51 question items. The first section of the questionnaire consisted of five questions to assess the socio-demographic characteristics of the participants such as gender, age, level of education, employment status, and profession; another section of the questionnaire included 46 questions to determine their knowledge about the diagnosis and management of COVID-19, personal protection measures, risk perception, and attitude of EMS workers. Knowledge and attitude were assessed by asking two Multiple Choice Questions (MCQ), and the remaining 44 questions were assessed through five Likert-type scales use statements. The response scales use anchors such as 1 = Strongly Agree, 2 = Agree, 3 = Neutral, 4 = Disagree, 5 = Strongly Disagree.

## Statistical analysis

Statistical analysis was performed using SPSS for Windows version 23.0 (SPSS Inc., Chicago, Ill., USA). Continuous variables are expressed as mean  $\pm$  SD or median and interquartile ranges (IQR) according to whether they exhibited a Gaussian distribution. Categorical variables are expressed as proportions and/or percentages. The  $\chi^2$  test was performed to determine the associations between the categorical variables.

## RESULTS

This survey was sent to 400 EMS workers. Responses were received from 275 (68.8%) participants, including emergency medical technicians (n=108; 39.3%), paramedics (n=138; 50.2%), doctors (n=8; 2.9%), and ambulance drivers (n=21; 7.6%). The demographic characteristics of the participants are summarized in Table 1.

The main source of COVID-19 information was social media (n=120, 43.6%) and television (n=120, 43.6%), followed by training seminars (n=21, 7.6%), medical books and journals (n=8, 2.9%), and newspapers (n=6, 2.2%).

Table 2 demonstrates the items related to COVID-19 knowledge. The responders who were high school graduates had higher percentages of correct answers

**TABLE 1.** DEMOGRAPHIC CHARACTERISTICS OF EMS WORKERS.

| Variables   | Emergency medical technician<br>N = 108 (%) | Paramedics<br>N = 138 (%) | Doctors<br>N = 8 (%) | Ambulance drivers<br>N = 21 (%) |
|-------------|---|---------------------------|----------------------|---------------------------------|
| Age         |   |                           |                      |                                 |
| ≤29 years   | 89 (88.4%)                                  | 36 (26.1%)                | 6 (75%)              | 10 (47.6%)                      |
| 30-39 years | 18 (16.7%)                                  | 101 (73.2%)               | 2 (25%)              | 10 (47.6%)                      |
| 40-49 years | 1 (0.9%)                                    | 1 (0.7%)                  | -                    | 1 (4.8%)                        |
| Gender      |   |                           |                      |                                 |
| Male        | 39 (36.1%)                                  | 43 (31.2%)                | 6 (75%)              | 19 (90.5%)                      |
| Female      | 69 (63.9%)                                  | 95 (68.8%)                | 2 (25%)              | 2 (9.5%)                        |
| Experience  |   |                           |                      |                                 |
| 1-5 years   | 60 (55.6%)                                  | 1 (0.7%)                  | 7 (87.5%)            | 16 (76.2%)                      |
| 6-10 years  | 31 (28.7%)                                  | 46 (33.3%)                | 1 (12.5%)            | 4 (19%)                         |
| 10-15 years | 12 (11.1%)                                  | 70 (50.7%)                | -                    | 1 (4.8%)                        |
| 16-20 years | 5 (4.6%)                                    | 21 (15.3%)                | -                    | -                               |

**TABLE 2.** KNOWLEDGE ABOUT COVID-19 AMONG EMS WORKERS.

| Questions   | Strongly Disagree/<br>Disagree | Neutral    | Strongly Agree/<br>Agree |
|---|--------------------------------|------------|--------------------------|
| 1. COVID-19 can be fatal?   | 36 (13.1%)                     | 41 (14.9%) | 198 (72.0%)              |
| 2. Molecular tests can be used for the diagnosis of COVID-19  | 21 (7.6%)                      | 72 (26.2%) | 182 (56.2%)              |
| 3. Hands should be washed with soap and water for 20 seconds to prevent the spread of infection                                 | 99 (36.0%)                     | 5 (1.8%)   | 171 (62.2%)              |
| 4. The first-line therapy for COVID-19 is antibiotics   | 120 (43.6%)                    | 88 (32.0%) | 67 (24.4%)               |
| 5. A COVID-19 vaccine has started to be administered  | 213 (77.5%)                    | 46 (16.7%) | 16 (5.8%)                |
| 6. COVID-19 is resistant to sanitizers containing at least 70% alcohol  | 137 (49.8%)                    | 77 (28.0%) | 61 (22.2%)               |
| 7. The estimated Incubation period for COVID-19 is about 2-14 days  | 4 (1.4%)                       | 12 (4.4%)  | 259 (94.2%)              |
| 8. COVID-19 is more severe in those with underlying diseases (hypertension, diabetes, cancer...)                                | 6 (2.2%)                       | 4 (1.5%)   | 265 (96.3%)              |
| 9. Most patients infected with COVID-19 develop severe acute respiratory illness  | 3 (1.1%)                       | 10 (3.6%)  | 262 (95.3%)              |
| 10. Contact isolation measures should be taken by healthcare professionals while caring for patients with COVID-19              | 2 (0.7%)                       | 6 (2.2%)   | 267 (97.1%)              |
| 11. Droplet isolation measures should be taken by healthcare professionals while caring for patients with COVID-19              | 2 (0.7%)                       | 7 (2.5%)   | 266 (96.8%)              |
| 12. Airborne isolation measures should be taken by healthcare professionals while caring for patients with COVID-19             | 1 (0.4%)                       | 9 (3.3%)   | 265 (96.3%)              |
| 13. A surgical mask is sufficient during operations that do not cause aerosolization in contact with COVID-19 infected patients | 144 (52.4%)                    | 55 (20.0%) | 76 (27.6%)               |
| 14. N95 masks are required during operations that do not cause aerosolization in contact with COVID-19 infected patients        | 41 (14.9%)                     | 50 (18.2%) | 184 (66.9%)              |
| 15. A patient infected with COVID-19 should wear a surgical mask  | 83 (30.2%)                     | 54 (19.6%) | 138 (50.2%)              |
| 16. A patient infected with COVID-19 should wear a N95 mask   | 95 (34.5%)                     | 50 (18.2%) | 130 (47.3%)              |

than those with a bachelor's degree ( $p < 0.001$ ). Their knowledge also differed significantly according to work experience. Participants with less than 10 years of experience had much more wrong answers than the ones with more than 10 years of experience ( $p < 0.001$ ).

In the section on the attitude of healthcare workers, there were 16 items. Table 3 summarized the attitude and preventive behaviors of EMS workers toward COVID-19.

## DISCUSSION

Healthcare professionals who are fully aware and knowledgeable about COVID-19 are essential for keeping health systems active and controlling the outbreak. It is very important to know EMS's knowledge of and attitudes toward the pandemic since they fight COVID-19 at the forefront and the risk of health personnel getting infected is high when necessary precautions are not taken. On the 29<sup>th</sup> of April, the Republic of Turkey Ministry of Health declared that 7,428 health



**TABLE 3.** ATTITUDE AND PREVENTIVE BEHAVIORS OF EMS WORKERS TOWARD COVID-19.

| Questions  | Strongly Disagree/<br>Disagree | Neutral     | Strongly Agree/<br>Agree |
|--|--------------------------------|-------------|--------------------------|
| 1. The prevalence of COVID-19 can be prevented by applying the universal recommendations given by the Turkey Ministry of Health and WHO    | 3 (1.1%)                       | 63 (22.9%)  | 209 (76.0%)              |
| 2. The transmission of COVID-19 to healthcare staff can be reduced by applying the precautions of the hospital infection control committee | 9 (3.3%)                       | 30 (10.9%)  | 236 (85.8%)              |
| 3. Patients with suspected or confirmed COVID-19 should be kept in isolation   | 0 (0.0%)                       | 4 (1.5%)    | 271 (98.5%)              |
| 4. I have sufficient knowledge about protective measures against COVID-19  | 23 (8.4%)                      | 67 (24.4%)  | 185 (67.2%)              |
| 5. I think that an in-hospital training program on COVID-19 is beneficial  | 35 (12.7%)                     | 85 (30.9%)  | 155 (56.4%)              |
| 6. Health professionals should have adequate knowledge about COVID-19  | 3 (1.1%)                       | 4 (1.5%)    | 268 (97.5%)              |
| 7. All patients with a confirmed diagnosis of COVID-19 should be admitted to the intensive care unit                                       | 194 (70.5%)                    | 33 (12.0%)  | 48 (17.5%)               |
| 8. Gargling or nose wash with saltwater protects against COVID-19  | 114 (41.5%)                    | 89 (32.4%)  | 72 (26.1%)               |
| 9. Consuming bountiful soup is effective in the prevention of COVID-19   | 97 (35.3%)                     | 85 (30.9%)  | 93 (33.8%)               |
| 10. Antiviral prophylaxis against COVID-19 should be required  | 117 (42.5%)                    | 103 (37.5%) | 55 (20.0%)               |
| 11. Vitamin C supplements were effective in the prevention of COVID-19   | 25 (9.1%)                      | 79 (28.7%)  | 171 (62.2%)              |
| 12. Vitamin D supplements were effective in the prevention of COVID-19   | 40 (14.5%)                     | 97 (35.3%)  | 138 (50.2%)              |
| 13. Consuming a mixture of honey and propolis is effective in the prevention of COVID-19   | 33 (12.0%)                     | 102 (37.1%) | 140 (50.9%)              |
| 14. I am worried about being infected with COVID-19  | 42 (15.3%)                     | 34 (12.4%)  | 199 (72.4%)              |
| 15. I feel under pressure as I can carry COVID-19 to my family   | 8 (2.9%)                       | 10 (3.6%)   | 257 (93.5%)              |
| 16. Social isolation is negatively affecting my mental health  | 59 (21.4%)                     | 42 (15.3%)  | 174 (63.3%)              |

workers had been infected with COVID-19. This number corresponds to 6.5% of the total COVID-19 cases in Turkey<sup>12</sup>. In this study, various topics such as level of knowledge about COVID-19, personal protection measures, risk perception, and attitude of EMS workers were explored. To the best of our knowledge, this is the first study assessing these topics in EMS workers on COVID-19 infection.

This survey was sent to 400 EMS workers and 275 (response rate 69%) participants answered the questionnaire. This rate is lower than similar surveys that have been conducted before. The low participation might be attributed to the overload of stressful work of the EMS staff.

In our study, social media and television were the main sources of participant's knowledge (88%). This result is compatible with previous studies<sup>10,13</sup>. A study by Prescott K. showed that the role of social media in providing information about COVID-19 was rather low in England (19%)<sup>14</sup>. This low rate might be due to the fact that the study was conducted at a time when the cases in the UK were just emerging.

In our study, the reason why social media is the main tool as a source of information may be that it is more accessible and practical than other online resources such as scientific journals and books. Moreover, the 'principle of least effort' might direct people to social media as a source of knowledge. On the other

hand, the spread of fake science via social media has been demonstrated, considering that 'The social media panic moved faster than the COVID-19 outbreak'<sup>15,16</sup>. Previous studies demonstrated that at least 40% of information shared on social media is fake, of which 20% is "dangerously" fake<sup>17</sup>. As a result of such issues, the WHO had to take some actions to guarantee that this virus would not spark a critical social media infodemic" which is triggered by wrong information<sup>18</sup>.

One of the most important topics that EMS workers should know is hand hygiene and antisepsis. Ensuring hand hygiene all the time is regarded as the most effective preventive measure against infection<sup>19</sup>. In this study, 36% of the responders were unaware of the correct hand washing or rubbing technique. In addition, we found that 78% of the participants had poor knowledge about floor and surface disinfection. There was also a significant variation in correct replies among participants with different educational levels. Responders who were high school graduates answered questions correctly with higher percentages than those with a bachelor's degree, probably because they are less well aware of the topic ( $p < 0.001$ ). Their knowledge also differed significantly according to work experience. Participants having less than 10 years of experience had much more wrong answers than the ones with more than 10 years of experience ( $p < 0.001$ ). Based on these results, we concluded that

the knowledge gap among EMS workers about hand hygiene and surface disinfection should be eliminated in the case of a next potential pandemic. Thus, standardized postgraduate education programs with a module focused on infection prevention, such as hand hygiene, or evidence-based strategies for the prevention of a specific type of infection are crucial.

Contact and droplet precautions should be always applied by healthcare workers caring for patients with COVID-19. Furthermore, airborne precautions should be implemented for aerosol-generating procedures. National COVID-19 guidelines for infection prevention and control suggest that wearing a surgical mask is efficient during the transport of suspected COVID-19 patients to the referral health care facility. However, EMS workers who are providing direct care to COVID-19 patients in settings where aerosol-generating procedures are frequently in place should wear N95, FFP2, or FFP3 masks<sup>20</sup>.

Despite national guideline suggestions, most of the respondents who participated in our study chose to raise the level of prevention measures despite the national guideline suggestions. 52% of the EMS workers agreed that a surgical mask is not enough during the procedures that do not generate aerosol. Moreover, a significant proportion of the participants perceived that a N95 mask is required (66%). However, a recent study demonstrated that 85% of healthcare workers in Iran believed that a surgical mask is effective for the prevention of COVID-19 on the procedures that do not generate aerosols. The difference of findings between the two studies may be related to several fake news on Turkish media that indicated that surgical masks were ineffective against COVID-19. This conflicting information may lead to anxiety and distress for healthcare workers. This situation develops unfavorable mental health outcomes that might affect their decisions on proper care. Thus, in our study, 20% of respondents who were frontline workers against COVID-19 had no idea about wearing an appropriate mask.

Currently, there are no approved curative treatments or a vaccine to prevent or treat COVID-19. There are lots of news that have not been based on scientific evidence, such as that dietary supplements are effective in preventing COVID-19, which has been featured in social media. Therefore, people are looking for alternative nutritional supplements to strengthen their immune system in order to protect themselves from this virus. In our study, 62% of the EMS workers agreed that Vitamin C supplements were effective in

preventing COVID-19. Furthermore, 50% of respondents believed that taking Vitamin D supplements were mandatory to protect themselves from COVID-19. Previous studies showed that a high dose of Vitamin C administration might be associated with a lower incidence of pneumonia in restricted population groups<sup>21</sup>. However, two recently published open-label studies relating to the use of vitamin C in different types of infections, associated with septic shock and acute respiratory distress syndrome (ARDS), demonstrated that using Vitamin C as a monotherapy or in combination with hydrocortisone, and thiamine had provided no significant benefit<sup>22,23</sup>. Thus, there is no robust scientific evidence to support the use of this vitamin in the management of COVID-19.

There is a limited number of studies on the use of vitamin D in COVID-19. Some of these reported that higher vitamin D3 doses might be useful for treating people who become infected with COVID-19<sup>24,25</sup>. However, they indicated that randomized controlled trials and large population studies should be conducted to evaluate this recommendation. Thus, Vitamin D supplementation is mentioned as a potentially beneficial treatment for COVID-19 infection but, on a scientific basis, with a low level of evidence until now. Even if there has been no approved effectiveness of these products, people's instincts have led them to take some actions such as taking nutritional supplements.

In order to defend themselves from COVID-19, a significant proportion of participants agreed that gargling with salty water (56%), drinking bounteous soup (64%), and eating a honey and propolis mixture (88%) can be protective. Since the beginning of this pandemic, some television programs in our country have argued that such suggestions will help to protect from this disease. Although there is no scientific evidence, these suggestions might have affected our participants and caused information chaos about this disease.

Although the national guidelines do not suggest antiviral prophylaxis against COVID-19, 57% of the participants agreed that using prophylactic antiviral drugs were required for the prevention of COVID-19. We perceived that the news telling that these protective drugs are effective on this virus has caused medical personnel to take such drugs thinking of preserving their lives. One-third of the participants thought that they do not have enough knowledge about such protective treatments. In addition, 40% of the participants declared that they have suffered a shortage of protective equipment when caring for COVID-19 patients. Based on

these data, it can be concluded that EMS workers need more often and more practical professional training.

Some limitations should be noted when interpreting our findings. The small sample size is the main strength of the study. The relatively low response rate from EMS staff, sample clustering, and the cross-sectional design of the study are regarded as limitations.

## CONCLUSION

As a consequence, although emergency workers have sufficient basic knowledge about COVID-19, there is a need for postgraduate training in many subjects. Professional organizations and non-governmental organizations should cooperate in this regard.

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## Author's Contribution

A.V. planned the methodology and wrote the manuscript; E.G. was responsible for data management and reporting collected data; A.Ö. provided access to crucial research components (personnel, equipment, environment), and was responsible for the Ethical Approval process; F.A.K. was responsible for the statistical interpretation and conclusion of the results; O.K. built the hypothesis of the article and reviewed the article scientifically, besides its spelling and grammar, before submission.

## RESUMO

**OBJETIVO:** Um bom conhecimento sobre a doença por coronavírus 2019 (COVID-19) entre profissionais de saúde é essencial para manter os sistemas de saúde ativos e controlar o surto. Nosso objetivo foi investigar o conhecimento e as atitudes dos funcionários do serviço médico de emergência (EMS) que lutam com o COVID-19 na vanguarda.

**MÉTODOS:** Um total de 400 trabalhadores do SME (médicos, enfermeiros, técnico de emergência médica, paramédicos e motoristas de ambulância) foram incluídos neste estudo. Conhecimento, atitude e comportamentos preventivos para COVID-19 foram avaliados usando um questionário on-line.

**RESULTADOS:** Um total de 275 trabalhadores do SME participou do estudo com uma taxa de resposta de 68,8%. Os entrevistados relataram que as maiores fontes comuns de conhecimento sobre COVID-19 foram as mídias sociais e a televisão (n = 240, 88%). No geral, > 96% dos participantes tinham conhecimento adequado sobre as rotas de transmissão do COVID-19. Entre os entrevistados, 36% deles desconheciam a técnica correta de lavar ou esfregar as mãos. Além disso, 78% dos participantes tinham pouco conhecimento sobre desinfecção de pisos e superfícies. A maioria dos participantes exibiu atitudes imprecisas em relação ao uso de equipamentos preventivos pessoais. Mais da metade dos trabalhadores do SME (52%) concordou que a máscara cirúrgica não é suficiente durante os procedimentos que não geram aerossol. Além disso, uma proporção significativa dos participantes (66%) percebeu que a máscara N95 é necessária.

**CONCLUSÕES:** Como consequência, embora os trabalhadores de emergência tenham conhecimento básico suficiente sobre o COVID-19, há necessidade de treinamento de pós-graduação em muitas disciplinas.

**PALAVRAS-CHAVE:** doença de coronavírus 2019, serviço médico de emergência, conhecimentos e atitudes, questionare

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# Polymorphisms of the GSTT1 and GSTM1 genes in polycystic ovary syndrome

 Mariana Mendes Porto Azevedo<sup>1</sup>  
 Alessandra Bernadete Trovó de Marqui<sup>2</sup>  
 Bruna Tavares Bacalá<sup>3</sup>  
 Marly Aparecida Spadotto Balarin<sup>4</sup>  
 Elisabete Aparecida Mantovanni Rodrigues de Resende<sup>5</sup>  
 Marco Fábio Prata Lima<sup>6</sup>

1. Mestre em Biociências pela Universidade Federal do Triângulo Mineiro/UFTM, Uberaba, MG, Brasil.
2. Professora do Instituto de Ciências Biológicas e Naturais/ICBN da UFTM, Uberaba, MG, Brasil.
3. Mestre em Genômica e Imunobiologia Aplicadas à Saúde Pública pela Escola de Enfermagem de Ribeirão Preto da Universidade de São Paulo (EERP/USP), Ribeirão Preto, SP, Brasil.
4. Professora do ICBN da UFTM, Uberaba, MG, Brasil.
5. Professora do Instituto de Ciências da Saúde/ICS da UFTM, Uberaba, MG, Brasil.
6. Professor do ICS da UFTM, Uberaba, MG, Brasil.

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## SUMMARY

**BACKGROUND:** This study aimed to investigate the deletion polymorphisms of the genes of the glutathione S-transferase family GSTT1 and GSTM1 in patients with Polycystic Ovarian Syndrome (POS), comparing them with a control population.

**METHODS:** Blood was collected from 219 women (110 with POS and 109 controls) and genomic DNA was extracted. For the analysis of polymorphisms, the technique used was multiplex PCR. In the statistical analysis, the chi-square test and multiple logistic regression were used.

**RESULTS:** There is no association between the GSTM1 null and GSTT1 null genotypes with PCOS when analyzed separately ( $P = 0.616$  and  $P = 0.188$ ). The analysis of the combined genotypes showed differences between the groups ( $P < 0.05$ ), evidencing that the genotypic combination GSTT1 positive and GSTM1 negative is more frequent among patients. In the multivariate analysis, smoking was more frequent in the control group (OR = 0.22; 95% CI - 0.87-0.57;  $P = 0.002$ ) while the presence of a family history of POS (OR = 2, 96; 95% CI - 1.54-5.68;  $P = 0.001$ ) was more frequent in women with POS.

**CONCLUSIONS:** In the studied sample, the deletion polymorphisms of the GSTT1 and GSTM1 genes isolated are not associated with POS, but in combination, they may be implicated in the etiology of the condition.

**KEYWORDS:** Multiplex Polymerase Chain Reaction. Polycystic Ovary Syndrome. Polymorphism, Genetic.

## INTRODUCTION

Polycystic ovary syndrome (POS) is a very common endocrine-metabolic disorder that affects women of reproductive age (18 to 44 years) and one of the main causes of female infertility. Its diagnosis is based

on the Rotterdam criteria, i.e., is characterized by the presence of at least two of the following criteria: chronic anovulation, clinical and/or biochemical signs of hyperandrogenism, and polycystic ovaries,

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CORRESPONDING AUTHOR: Mariangela Torreglosa Ruiz Cintra  
 Instituto de Ciências Exatas, Naturais e Educação (Icene) – Universidade Federal do Triângulo Mineiro (UFTM)  
 Av. Dr. Randolpho Borges Jr., 1400, Uberaba, MG, Brasil – 38064-200  
 Tel: +55 34 3331-3143  
 E-mail: mariangela.cintra@uftm.edu.br



characterized by the ultrasonographic examination. High levels of androgens cause hirsutism and acne. There is some insulin resistance that leads to obesity and diabetes type 2<sup>1</sup>.

The syndrome has a multifactorial character; therefore, genetic factors play an important role in its etiology, with emphasis on single-nucleotide polymorphisms (SNPs)<sup>1</sup>. In addition to genetic predisposition, oxidative stress can contribute to the pathogenesis of POS, and oxidative stress parameters can be used as biomarkers for the early diagnosis and screening of high-risk groups<sup>2,3</sup>.

Figure 1 illustrates how oxidative stress plays a key role in the physiopathology of POS<sup>3</sup>. The family of Glutathione S-transferase enzymes is involved in the detoxification of reactive oxygen species, which are generated in large quantities during oxidative stress. In this sense, polymorphisms in genes of this family can alter the functions of enzymes that act on the metabolism of these molecules and, consequently, be associated with the clinical conditions of POS. Such polymorphisms reduce or eliminate the detoxification activities of these enzymes and could increase people's susceptibility to diseases, including POS<sup>4</sup>.

Genes *GSTM1* and *GSTT1*, located at 1q13.3<sup>5</sup> and 22q11.2<sup>6</sup>, respectively, belong to the family of GST. Polymorphisms of complete deletion of these genes have already been extensively investigated in type 2 diabetes mellitus (DMT2), a clinical consequence of POS<sup>7-13</sup>. However, only two studies have evaluated the polymorphisms of genes *GSTT1* and *GSTM1* in POS<sup>14,15</sup>.

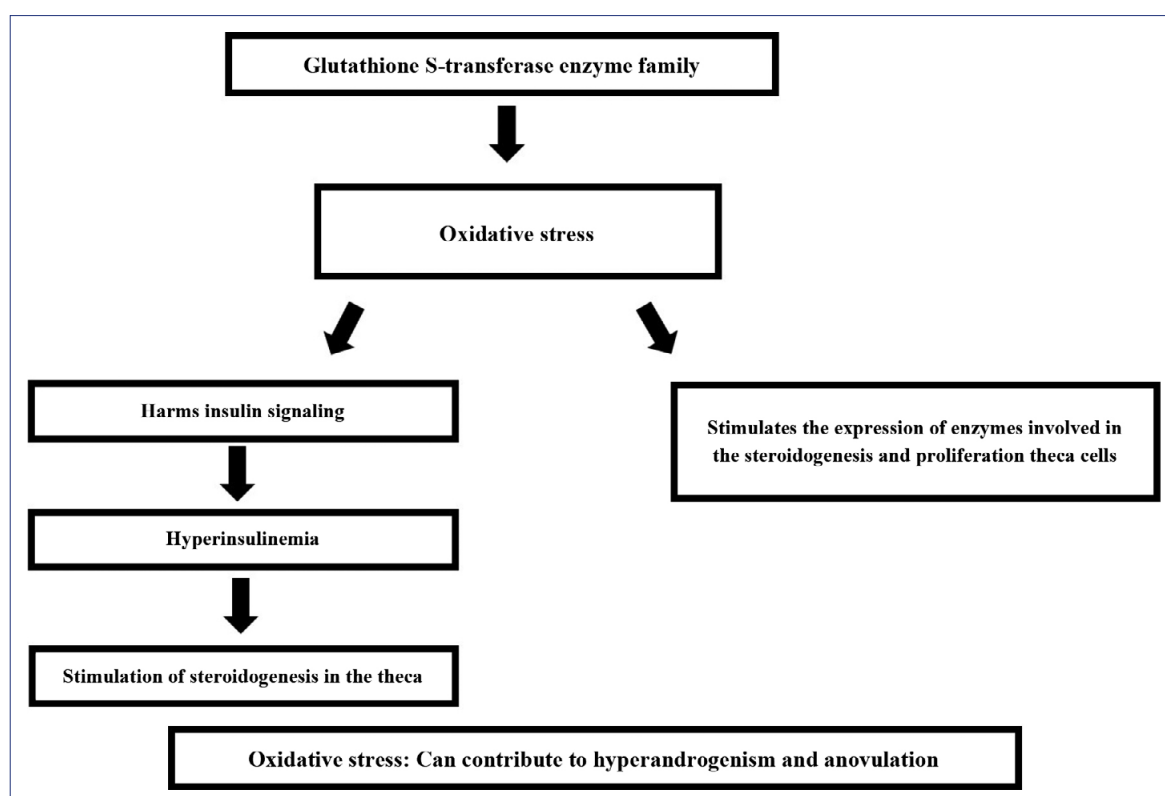
Thus, based on the evidence presented, the objective of this study was to investigate the deletion polymorphisms of the genes *GSTT1* and *GSTM1* in patients with a POS and in a control group.

## METHODS

The project was approved by the Research Ethics Committee (CEP) of the Federal University of Triângulo Mineiro (UFTM), under protocol number 1,796, and all participants signed the Informed Consent Form.

The study had the participation of 219 women (110 patients with POS and 109 controls), who were being followed-up in the outpatient clinic of General Gynecology, Endocrine Gynecology, and Endocrinology

**FIGURE 1.** FLOWCHART ILLUSTRATING THE ROLE OF THE GLUTATHIONE S-TRANSFERASE ENZYME FAMILY IN OXIDATIVE STRESS, A PROCESS THAT CAN CONTRIBUTE TO HYPERANDROGENISM AND ANOVULATION, ALL CLINICAL CHARACTERISTICS OF POS.



of the Maria da Glória Outpatient Clinic, of UFTM, Uberaba, MG, Brasil, at the moment of the study, constituting a sample by convenience. The patients with POS were diagnosed with the condition based on the Rotterdam criteria, and the control group was comprised of women of reproductive age, without a history or sign of POS. As the exclusion criteria, we did not evaluate patients with Cushing's syndrome, 21-hydroxylase deficiency, thyroid dysfunction, hyperprolactinemia, diabetes, androgen-secreting tumors, antiandrogens, statins, glucocorticoids, or infertility medications.

We collected 8 mL of peripheral blood, in vacuum tubes with EDTA; the DNA was extracted using the salting-out technique<sup>16</sup>.

The analysis of the deletion polymorphisms of genes *GSTM1* and *GSTT1* was carried out using the multiplex PCR technique. The primers for the polymorphism of the gene *GSTM1* were sense: 5' GAA CTC CCT GAA AAG CTA AAG C 3' and antisense: 5' GTT GGG CTC AAA TAT ACG GTG G 3' (219 pb) and of gene *GSTT1* sense: 5' TTC CTT ACT GGT CCT CAC ATC TC 3' and antisense: 5' TCA CCG GAT CAT GGC CAG CA 3' (480 pb). The sequence of primers for gene *CYP11A1* was sense: 5' GAA CTG CCA CTT CAG CTG TCT 3' and antisense: 5' CAG CTG CAT TTG GAA GTG CTC 3' (312 pb), and this gene was used as an internal positive control for the reaction.

The PCR reaction was carried out on a final volume of 30 µL, containing 0.2 mM dNTP, 1x PCR buffer, 3 pmol of each primer, 1 unit of Taq DNA Polymerase, and approximately 100 ng of genomic DNA. The PCR amplification consisted of an initial stage at 94 °C for 5 minutes, followed by 35 cycles at 94 °C for 2 minutes (denaturation), 59 °C for 1 minute (annealing), 72 °C for 1 minute (extension), and a final extension at 72 °C for 10 minutes. The PCR products were applied in 1.5% agarose gel, stained using GelRed™, and analyzed under ultraviolet light for genotyping.

We used the chi-square test to compare data from the case and control groups. The multiple logistic regression model was used to determine the effects of risk factors (smoking, family history of POS, alcoholism, and presence of polymorphisms) in POS. The results of the logistic regression analysis were presented in odds-ratio (OR) and with a confidence interval of 95% (95% CI). For all analyzes, the level of significance was set at 5% ( $p \leq 0.05$ ).

## RESULTS

The mean age of the patients and the control group was 26 years ( $\pm 7.54$ ) and 31 years ( $\pm 9.25$ ), respectively. Regarding the risk factors, the data show that 13.8% of the participants are smokers, something that was more frequent in the control group. With regard to alcoholism, it was observed that 27.8% of the women studied had this habit, but no significant differences were found between the groups. Observing the family history of POS, 29.3% of the participants had relatives with POS, with a significant difference between the groups, and the patients had a greater number of relatives with POS (67.6%) compared to the control group (32.4%).

In Table 1 it is possible to see the frequency of the deletion polymorphisms of genes *GSTT1* and *GSTM1* in women with POS and in the control group. No significant differences were observed between the groups when they were analyzed individually.

**TABLE 1.** GENOTYPIC DISTRIBUTION OF POLYMORPHISMS *GSTT1* AND *GSTM1* IN PATIENTS WITH POS AND CONTROLS.

| Genes        | Total<br>n (%) | Case<br>n (%) | Control<br>n (%) | P     |
|--------------|----------------|---------------|------------------|-------|
| <i>GSTT1</i> | 219 (100)      | 110 (50.2)    | 109 (49.8)       | 0.616 |
| [+]          | 162 (100)      | 83 (51.2)     | 79 (48.8)        |       |
| [-]          | 57 (100)       | 27 (47.4)     | 30 (52.6)        |       |
| <i>GSTM1</i> | 219 (100)      | 110 (50.2)    | 109 (49.8)       | 0.188 |
| [+]          | 129 (100)      | 60 (46.5)     | 69 (53.5)        |       |
| [-]          | 90 (100)       | 50 (55.6)     | 40 (44.4)        |       |

Note: Values of  $P \leq 0.05$  are significant; [+]: Presence of the gene; [-]: Deletion of the gene.

The analysis of the combined genotypes showed differences between the group of patients and the controls ( $\chi^2=11.534$ ,  $p<0.05$ ) (Table 2). This analysis shows that the combination of genotypes *GSTT1* +/*GSTM1*- is more frequent in patients.

**TABLE 2.** FREQUENCY OF THE GENOTYPES *GSTT1* AND *GSTM1* COMBINED IN PATIENTS AND CONTROLS.

| Genotypes                      | Patient<br>n (%) | Control<br>n (%) | P     |
|--------------------------------|------------------|------------------|-------|
|                                |                  |                  | <0.05 |
| <i>GSTT1</i> +/ <i>GSTM1</i> + | 44 (40)          | 52 (47.7)        |       |
| <i>GSTT1</i> +/ <i>GSTM1</i> - | 38 (34.5)        | 17 (15.6)        |       |
| <i>GSTT1</i> -/ <i>GSTM1</i> + | 16 (14.5)        | 27 (24.8)        |       |
| <i>GSTT1</i> -/ <i>GSTM1</i> - | 12 (10.9)        | 13 (11.9)        |       |

Note: Values of  $P \leq 0.05$  are significant; [+]: Presence of the gene; [-]: Deletion of the gene.

The multivariate analysis carried out to determine the effects of risk factors (smoking, family history of POS, alcoholism, and presence of polymorphisms) in POS showed that smokers were more frequent in the control group (odds ratio=0.22; CI 95% - 0.87-0.57;  $p=0.002$ ), while a family history of POS (OR=2.96; 95% CI: 1,54-5,68;  $p=0.001$ ) was more frequent among women with the syndrome, with a significant difference between the groups. The variables of alcoholism and deletion polymorphisms of genes *GSTT1* and *GSTM1* presented no statistically significant differences between the groups analyzed.

## DISCUSSION

Glutathione S-transferases (GSTs) are a superfamily of detoxifying enzymes involved in maintaining cellular integrity, oxidative stress, and protection against DNA damage. Many polymorphisms have been reported to occur in GST coding genes, which can reduce their efficiency and increase the risk of certain human diseases, such as cancer<sup>4</sup>.

A study explored the association between POS and oxidative stress and examined the relationship between biomarkers of oxidative stress and insulin parameters, concluding that oxidative stress may be a contributing factor for future risk of cardiovascular disease in these women, in addition to known characteristics such as dyslipidemia and obesity<sup>17</sup>. Besides obesity, there is evidence that sex hormones, particularly estradiol, in POS can contribute to increased oxidative stress<sup>2</sup>.

In the present study, no association was found between the deletion of genes *GSTT1* and *GSTM1* and POS in isolation, only in combination. A previous study suggests that the combined effect of *CYP1A1* and the mutant genotypes *GSTM1* and *GSTT1* confers an increased risk of the syndrome in Indian women<sup>14</sup>. Another study analyzed parameters of oxidative stress and GST polymorphisms in non-obese adolescents, with normal levels of insulin, and recently diagnosed with SOP. Regarding the molecular data, girls with POS, carriers of the null *GSTM1* genotype showed significantly lower testosterone in comparison with those carriers of the active *GSTM1* genotype<sup>15</sup>.

Another study investigated the four most common polymorphisms of GST (*GSTM1*, T1, A1, and P1) in epithelial ovarian cancer and found that these can affect both the susceptibility to and the progression of this type of cancer<sup>18</sup>.

Polymorphisms in genes *GSTT1* and *GSTM1* were analyzed in gynecological conditions such as endometriosis<sup>19-22</sup> and pre-eclampsia<sup>23,24</sup>, with divergent results (Table 3), even in populations of the same country of origin. The discrepancy between the results may be due to the particularities of the samples investigated in regarding their ethnicity and genetic background. It is also important to highlight that, in addition to POS, endometriosis is one of the main causes of female infertility.

**TABLE 3.** STUDIES THAT EVALUATED POLYMORPHISMS IN THE GENES *GSTT1* AND *GSTM1* IN GYNECOLOGICAL CONDITIONS AND THE PRESENCE (YES) OR NO PRESENCE OF ASSOCIATION, ACCORDING TO THE CONDITION INVESTIGATED.

| Study/Country of origin                               | Sample                                       | Association                             |
|---|--|---|
| Henidi et al. <sup>19</sup> /Tunisia                  | 105 women with endometriosis<br>150 controls | Yes                                     |
| Kubiszkeski et al. <sup>20</sup> /Brasil              | 121 women with endometriosis<br>97 Controls  | No - <i>GSTM1</i><br>Yes - <i>GSTT1</i> |
| Tuo et al. <sup>21</sup> /China                       | 262 women with endometriosis<br>275 controls | No                                      |
| Batista et al. <sup>22</sup> /Brasil                  | 49 women with endometriosis<br>83 controls   | No                                      |
| Guan et al. <sup>23</sup> /China                      | 232 women with pre-eclampsia<br>293 controls | Yes                                     |
| Sljivancanin Jakovljevic et al. <sup>24</sup> /Serbia | 50 women with pre-eclampsia<br>50 controls   | Yes                                     |

DMT2 is highly prevalent in patients with POS<sup>25</sup>. Seven studies investigated the polymorphisms in the genes *GSTT1* and *GSTM1* in patients with DMT2 and positively associated them with the condition, either individually or in combination<sup>7-13</sup>. One of these studies was carried out with 120 Brazilian patients with DMT2 and it suggests that the null *GSTM1* and *GSTT1* genotypes may contribute to the clinical course of the disease<sup>10</sup>. A meta-analysis of 25 studies concluded that the null *GSTM1* and *GSTT1* genotypes increase the risk of DMT2 alone, in combination, or with respect to ethnicity<sup>26</sup>.

In this study, the multiple logistic regression model showed an increased frequency of smokers in the control group, which may be a peculiar characteristic of the sample. The increased frequency of POS in the study group is associated with the multifactorial nature of the condition, which demonstrates the contribution of genetic factors in its etiology.

Recently, a multicenter study was published in Brasil that could guide public strategies specific for primary and secondary prevention of metabolic and

reproductive comorbidities in the population with POS in the country. This information is extremely relevant in view of the extension of our country associated with a population of mixed ethnic origin and marked variation of diet and cultural characteristics. These characteristics provide a unique opportunity to investigate the association of factors related to lifestyle, environment, genetics, epigenetics, and the phenotypic expression of POS<sup>27</sup>.

One limitation of our study was the sample analyzed, considered small for research with genetic polymorphisms. However, the scarcity of studies published on polymorphisms in the genes *GSTM1* and *GSTT1* in POS compromised the comparison with the results found herein but emphasizes the need for additional studies regarding the contribution of these polymorphisms to the development of this endocrinopathy.

It is also important to highlight that the lack of association in our study may be due to the ethnicity and genetic background of the population investigated. Another interesting approach, in subsequent studies, would be to examine whether there is an association between comorbidities such as diabetes type 2, central obesity, and cardiovascular disease and the polymorphisms analyzed. However, this would require dividing the patients into subgroups, which might not have statistical significance.

It is also worth mentioning that there are environmental, behavioral, and psychological factors that can

interfere with the phenotype of POS<sup>28</sup>, and that the sample does not reflect the community, since they were patients who sought care. Therefore, further studies are needed in order to minimize this sample bias. Thus, the lack of association of the gene polymorphisms studied and the syndrome should also be explored.

## CONCLUSIONS

The deletion polymorphisms of genes *GSTT1* and *GSTM1* in isolation are not associated with POS; however, in combination, they may be involved in the etiology of the condition in the sample investigated.

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## Author's Contribution

Mariana Mendes Porto Azevedo, Alessandra Bernadette Trovó de Marqui: concept, data collection and analysis, writing; Bruna Tavares Bacalá, Marly Aparecida Spadotto Balarin, Marco Fábio Prata Lima, Mariana Kefalas Oliveira Gomes: data collection and analysis.

## RESUMO

**OBJETIVO:** Este estudo teve como objetivo investigar os polimorfismos de deleção dos genes da família glutathione S-transferase *GSTT1* e *GSTM1* em pacientes com síndrome dos ovários policísticos (SOP), comparando-as com uma população controle.

**MÉTODOS:** Foi colhido sangue de 219 mulheres (110 com SOP e 109 controles) e extraído o DNA genômico. Para análise dos polimorfismos, a técnica empregada foi PCR multiplex. Na análise estatística foi utilizado o teste do qui-quadrado e regressão logística múltipla.

**RESULTADOS:** Não há associação dos genótipos *GSTM1* nulo e *GSTT1* nulo com SOP quando analisados isoladamente ( $p=0,616$  e  $p=0,188$ ). A análise dos genótipos combinados mostrou diferenças entre os grupos ( $p<0,05$ ), evidenciando que a combinação genotípica *GSTT1* positivo e *GSTM1* negativo é mais frequente entre as pacientes. Na análise multivariada, o hábito tabagista foi mais frequente no grupo controle (OR=0,22; IC 95% - 0,87-0,57;  $p=0,002$ ), enquanto que a presença do histórico de SOP familiar (OR=2,96; IC 95% - 1,54-5,68;  $p=0,001$ ) foi mais frequente nas mulheres com SOP.

**CONCLUSÕES:** Na casuística estudada, os polimorfismos de deleção dos genes *GSTT1* e *GSTM1* isolados não estão associados a SOP, mas em combinação podem estar implicados na etiologia da condição.

**PALAVRAS-CHAVE:** Reação em cadeia da polimerase multiplex. Síndrome do ovário policístico. Polimorfismo genético.

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# Infliximab-induced remission improves physical activity in patients with active Crohn's disease


 Fernando de Azevedo Lucca<sup>1</sup>

 Carla Malaguti<sup>3</sup>


 Liliana Andrade Chebli<sup>1</sup>


 Maycon Moura Reboredo<sup>2,3</sup>


 Bruno Valle Pinheiro<sup>2</sup>

 Tarsila Campanha da Rocha Ribeiro<sup>1</sup>

 Felipe Meirelles de Azevedo<sup>3</sup>

 José Otávio do Amaral Corrêa<sup>1</sup>

 Pedro Duarte Gaburri<sup>1</sup>

 Julio Maria Fonseca Chebli<sup>1</sup>

**1.** Unidade de Gastroenterologia, Departamento de Medicina, Centro de Doenças Inflamatórias Intestinais, Hospital Universitário da Universidade Federal de Juiz de Fora, Faculdade de Medicina da Universidade Federal de Juiz de Fora, Juiz de Fora, MG, Brasil.

**2.** Laboratório de Investigação Pulmonar, Universidade Federal de Juiz de Fora, Juiz de Fora, MG, Brasil.

**3.** Programa de Pós-Graduação em Ciências da Reabilitação e Desempenho Físico-Funcional da Universidade Federal de Juiz de Fora, Faculdade de Medicina da Universidade Federal de Juiz de Fora, Juiz de Fora, MG, Brasil.

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## SUMMARY

**AIM:** To compare the level of physical activity (PA), exercise capacity, and body composition before and after infliximab-induced clinical remission in patients with Crohn's disease (CD).

**METHODS:** This prospective longitudinal study evaluated 44 adult outpatients with active CD before infliximab administration and 24 weeks after infliximab therapy. The patients were evaluated for PA in daily life, exercise capacity, muscle strength, and body composition.

**RESULTS:** 38 (86.4%) patients achieved infliximab-induced remission at 24 weeks and presented an increment in the number of steps taken of 1092 (7440±2980 vs. 6348±3177, respectively;  $p=0.006$ ). The inactive time was reduced when compared to the baseline value (454.2±106.3 vs. 427.9±97.8, respectively;  $p=0.033$ ). There was no difference in the distance walked before and after infliximab therapy, while there was an increase in the fat mass index in responders to infliximab compared to the baseline (19.1±7.6 vs. 14.9±5.8;  $p=0.001$ ).

**CONCLUSIONS:** Infliximab-induced remission was shown to be effective for increasing physical activity by improving the number of steps and reducing inactive time. The maintenance of clinical remission associated with incentives to regular PA may contribute to making these patients reach an ideal level of PA.

**KEYWORDS:** Crohn disease. Inflammatory Bowel Diseases. Infliximab. Motor activity.

## INTRODUCTION

Crohn's disease (CD) is a chronic, transmural, and idiopathic inflammatory bowel disease (IBD) that can involve any segment of the gastrointestinal tract. The incidence of IBD and CD has been rising with an annual incidence ranging from 3 to 20 cases per

100,000. Its relapsing-remitting course can negatively impact patient social, educational, and professional activities, hence having a profound effect on their health-related quality of life (HRQOL)<sup>1</sup>.

During the last decades, the impact of physical

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CORRESPONDING AUTHOR: Julio Maria Fonseca Chebli

Rua Maria José Leal, 296- Juiz de Fora, MG, Brasil – CEP 36036-247 Tel / Fax: +55 (32) 21023841.

E-mail: chebli@globocom

activity (PA) on health has been recognized in several areas. Its role in healthy subjects is associated with an improvement of physical, psychosocial/social, and cognitive fields as well as an enhancement in HRQOL<sup>2</sup>. Moreover, PA has also been proven to increase HRQOL and reduce all-cause mortality even in patients with chronic diseases such as type 2-diabetes, arthritis, and cancer<sup>3</sup>. Thus, PA is considered a predictor of public health, and the definition of an individual as physically inactive has a critical impact further on patient management<sup>4</sup>.

Although the role of PA in IBD patients is not well defined, some studies about its effect on the disease's etiology and activity have addressed this issue<sup>5,6</sup>. IBD patients, mainly those with CD, seem to have a lower level of PA when compared to controls<sup>7</sup>. Tew et al.<sup>8</sup> evaluated 877 IBD patients and demonstrated that the majority of them were minimally active or inactive, and this sort of behavior was associated with disease activity. The lower PA level in this population can be explained by a multifactorial scenario. Disease manifestations (e.g., diarrhea, abdominal pain, sarcopenia, osteoporosis, and fatigue) associated with mood disorders may contribute to the sedentary lifestyle of these patients, resulting in the reduction of their physical performance<sup>9</sup>. Of note, the impact of PA on the immune function has been demonstrated in non-IBD populations, and it is hypothesized that it could be related to its anti-inflammatory effect by decreasing visceral fat tissue and releasing pro-inflammatory cytokines/myokines<sup>10</sup>.

Biological therapy represented a significant advance for CD treatment, reducing hospitalizations, surgical interventions, and achieving good rates of clinical remission of the disease<sup>11</sup>. Taking into account that a low level of PA may be associated with disease activity<sup>12</sup>, it seems very interesting to investigate it after infliximab (IFX)-induced remission. This study evaluated a cohort of patients with active CD submitted to IFX therapy in order to compare the exercise capacity and PA before and after IFX-induced clinical remission. It is hypothesized that CD patients who achieved IFX-induced remission present a significant improvement in the previous levels of exercise capacity and PA in daily life.

## METHODS

This prospective longitudinal study, with a before-and after-intervention, was conducted between March

2014 and June 2017, on adult outpatients with CD from the Inflammatory Bowel Disease Center at the University Hospital of the Federal University of Juiz de Fora, Brasil. The study was approved by the institutional Ethical Committee (number 95,125). All patients provided informed consent.

We included patients with CD, aged  $\geq 18$  years and under 65 years, with a 3-month history of active disease, defined as a Harvey-Bradshaw index (HBI) score  $\geq 5$ , with an indication for anti-TNF (anti-tumor necrosis factor- $\alpha$ ) therapy.

Exclusion criteria were: severe disease requiring hospitalization or immediate surgery, history of recent surgery, previous treatment with an anti-TNF agent or other biologic therapy, short-bowel syndrome, ostomy, class II or III obesity, severe hepatic disease, immunodeficiency syndromes, end-stage renal disease, neuromuscular diseases, malignancy, hemoglobin level below 11g/dL at baseline, participation in a formal exercise training program in the preceding 6 months.

Eligible patients received induction therapy consisting of intravenous injections of 5mg/Kg of IFX at weeks 0, 2, and 6, followed by maintenance infusion (5mg/Kg) at weeks 14 and 22. Participants were followed-up until week 24. Doses of concomitant medications were maintained constant except for those using steroids, for which the dose could be reduced.

Each one of the patients was evaluated at two different moments: M1, before IFX administration (baseline) and M2, 24 weeks after IFX therapy. The primary endpoint was an increase in the total number of steps/day in daily life. HBI, anthropometry and body composition, physical activity in daily life, exercise capacity, peripheral muscle strength, and Hospital Anxiety and Depression Scale (HADS) were assessed in all patients.

At entry, the eligibility criteria were assessed and medical history was recorded, including age, gender, smoking status, and body mass index. The disease-associated variables recorded were duration of disease, location and phenotype of CD according to the Montreal classification<sup>13</sup>, CD activity, current therapy, and CD-related surgical history. CD behavior was established by both ileocolonoscopy and computed enterography performed within six months prior to study entry. An HBI score  $\geq 5$  indicated active disease, while clinical remission after infliximab therapy was defined by a HBI less than 5<sup>14</sup>. For practical purposes, patients were categorized as non-responders to the IFX therapy if a failure to achieve clinical remission (i.e., HBI $\leq 4$ ) from baseline was registered following

anti-TNF therapy, as assessed at 24 weeks after the initial infusions.

Physical activity in daily life was monitored by DynaPort Activity Monitor (McRoberts BV, Netherlands) used at least four days in order to obtain the total number of steps/day, active time, and inactive time<sup>15</sup>. Anthropometry and body composition measurements for the assessment of lean mass and fat mass were obtained by electrical bioimpedance (Quantum BIA-101Q, Detroit, MI)<sup>16</sup>. Exercise capacity was evaluated by the Shuttle Walk Test (SWT)<sup>17</sup>, in which the total distance covered in meters indicates the exercise capacity. The handgrip strength (HS) was assessed by a hydraulic dynamometer (SAEHAN®, Korea) in Kg/f<sup>18</sup>. Anxiety and depression levels were assessed using the HADS, validated for the Brazilian population. According to the validated translations of the HADS, the cutoff value for depressive or anxiety symptoms was 8<sup>19</sup>.

### Statistical analysis

Statistical analysis was performed using SPSS20.0 (SPSS, Chicago, USA). The Wilcoxon test was used to non-parametric variables, which were described in medians and interquartile intervals. The paired t-test was used for evaluating parametric variables.

To detect this 20% difference in the number of steps taken by CD patients before and after Infliximab-induced remission, with 90% power and a 5% significance level using a one-sided test, the required sample size is at least 40 patients, considering an IFX-induced remission rate at 24 weeks of 70%. The number of patients was increased to 44 to compensate for an anticipated dropout rate of 10%.

Comparisons before and after infliximab-induced remissions on the same subjects were performed for PA, exercise capacity, peripheral muscle strength, LM and FM indices. This same analysis was carried out for patients categorized as primary non-responders to anti-TNF therapy. Statistical significance was set  $p < 0.05$ .

## RESULTS

A total of 50 adult CD patients were assessed for eligibility in the study. Among them, 3 (5.8%) patients were excluded because they presented indeterminate colitis, class III obesity, or contraindications to *anti-TNF therapy*. In addition, 3 (5.8%) subjects discontinued the study, 2 due to an adverse event, and 1 for missing

the follow-up. Consequently, 44 patients completed 24 weeks of the complete evaluation. The baseline characteristics of the participants are shown in Table 1. The mean ( $\pm$ SD) patient age was 39.9 ( $\pm$ 12.5), and 56.8% were females. The mean duration of the disease was 6.5 years. The most common location and phenotype of CD were Ileocolonic (61.4%) and stricturing (56.8%), respectively. Prior resections were performed in 31.8% of patients. At the baseline, 20.5% were under steroids and 86.4% thiopurines therapy. Of the total population, 38 (86.4%) patients achieved IFX-induced clinical remission at the end of the 24 weeks, while 6 (13.6%) were non-responders to anti-TNF.

*Patients who responded to the IFX treatment* did not differ on the baseline and during clinical remission at week 24 in terms of peripheral muscle strength and LM index. In contrast, there was a higher increase in the FM index in responders following IFX therapy compared to the baseline period ( $19.1 \pm 7.6$  vs.  $14.9 \pm 5.8$ ;  $p = 0.001$ ; Table 2). *Participants with CD refractory to anti-TNF therapy* did not present any significant change in body composition or skeletal muscle function after receiving IFX (Table 3).

**TABLE 1.** BASELINE DEMOGRAPHIC AND CLINICAL CHARACTERISTICS OF PATIENTS WITH CROHN'S DISEASE RECEIVING INFLIXIMAB (N=44)

| Characteristic                           |                              |
|--|------------------------------|
| Gender, (M:F)                            | 19:25                        |
| Age, (yr)*                               | 39.9 $\pm$ 12.5              |
| Race, n (%)                              |                              |
| White                                    | 36 (81.8)                    |
| Non-white                                | 8 (18.2)                     |
| Body-mass index, (kg/m <sup>2</sup> )*   | 22.4 $\pm$ 3.3               |
| Current smoker, n (%)                    | 12 (27.3)                    |
| Disease location, n (%)                  |                              |
| [L1/L2/L3]                               | 10 (22.7)/7 (15.9)/27 (61.4) |
| Disease behavior, n (%)                  |                              |
| [B1/B2/B3]                               | 11 (25)/25 (56.8)/8 (18.2)   |
| p (perianal disease)                     | 10 (22.7)                    |
| Disease duration, (yr)*                  | 6.5 $\pm$ 5.8                |
| HBI score*                               | 6.0 (2 – 18)                 |
| Previous intestinal resections, n (%)    | 14 (31.8)                    |
| Concurrent therapy at study entry, n (%) |                              |
| Glucocorticoids                          | 9 (20.5)                     |
| Thiopurines                              | 38 (86.4)                    |
| Glucocorticoids plus thiopurines         | 2 (4.5)                      |

\*- Mean  $\pm$  standard deviation. Abbreviations: L1 = ileal; L2 = colonic; L3 = ileocolonic; B1 = non-stricturing, non-penetrating; B2 = stricturing; B3 = penetrating; HBI = Harvey-Bradshaw Index

**TABLE 2.** EXERCISE CAPACITY, PHYSICAL ACTIVITY IN DAILY LIFE, AND BODY COMPOSITION IN CROHN'S DISEASE PATIENTS BEFORE AND AFTER INFLIXIMAB-INDUCED CLINICAL REMISSION (N=38)

| Variable*                         | Before IFX therapy (Active CD) | After IFX therapy (Inactive CD) | P      |
|-----------------------------------|--------------------------------|---------------------------------|--------|
| HADS-D 5                          | .62 ± 3.63                     | 3.81 ± 2.63                     | 0.007  |
| HADS-A                            | 7.48 ± 4.20                    | 4.97 ± 3.57                     | 0.001  |
| HBI score                         | 6.0 (1 – 18)                   | 1.58 (0 – 4)                    | <0.001 |
| Shuttle walking test (m)          | 532.2 ± 222.3                  | 586.5 ± 252.4                   | 0.10   |
| Body composition (Kg)             |                                |                                 |        |
| Lean mass index                   | 46.7 ± 8.5                     | 48.3 ± 10.8                     | 0.11   |
| Fat mass index                    | 14.9 ± 5.8                     | 19.1 ± 7.6                      | 0.001  |
| Isometric handgrip force (kgf)    | 33.8 ± 10.7                    | 34 ± 10.8                       | 0.92   |
| Physical activities in daily life |                                |                                 |        |
| Active time (min)                 | 237.8 ± 93.4                   | 255.6 ± 90.6                    | 0.09   |
| Inactive time (min)               | 454.2 ± 106.3                  | 427.9 ± 97.8                    | 0.033  |
| Steps/day                         | 6348 ± 3177                    | 7440 ± 2980                     | 0.006  |

\* Values are expressed as mean and standard deviation. Abbreviations: CD= Crohn's disease; IFX= infliximab; HBI = Harvey-Bradshaw Index.

**TABLE 3.** EXERCISE CAPACITY, PHYSICAL ACTIVITY IN DAILY LIFE, AND BODY COMPOSITION IN CROHN'S DISEASE PATIENTS NON-RESPONDERS TO INFLIXIMAB THERAPY (N=6)

| Variable*                         | Before IFX therapy (Active CD) | After IFX therapy (Inactive CD) | P    |
|-----------------------------------|--------------------------------|---------------------------------|------|
| HBI score                         | 8.50 (6 – 16)                  | 8.0 (1 – 13)                    | 0.85 |
| Shuttle walking test (m)          | 693.3 ± 369.5                  | 728.3 ± 294.3                   | 0.75 |
| Body composition (Kg)             |                                |                                 |      |
| Lean mass index                   | 48.1 ± 7.7                     | 48.5 ± 5.5                      | 0.82 |
| Fat mass index                    | 15.1 ± 5.6                     | 18.1 ± 8.6                      | 0.13 |
| Isometric handgrip force (kgf)    | 32.8 ± 12.2                    | 36.4 ± 9.8                      | 0.27 |
| Physical activities in daily life |                                |                                 |      |
| Active time (min)                 | 178.2 ± 84.4                   | 200.5 ± 77.2                    | 0.30 |
| Inactive time (min)               | 517.6 ± 106.3                  | 484.1 ± 106.1                   | 0.34 |
| Steps/day                         | 5084 ± 3875                    | 5436 ± 2784                     | 0.86 |

\* Values are expressed as mean and standard deviation. Abbreviations: IFX= infliximab; HBI = Harvey-Bradshaw Index

There was no difference in the distance walked in the SWT performed before and after anti-TNF therapy. At week 24, patients who achieved IFX-induced remission presented a significant increment from baseline in the number of steps taken of 1092 (7440±2980vs.6348±3177, respectively;  $p=0.006$ ). Of note, 18 out of 38 responder patients (47.3%) reached the mark of 7500 steps. Additionally, inactive time was reduced over clinical remission at week 24 compared to the baseline period (454.2±106.3vs.427.9±97.8, respectively;  $p=0.033$ ). Also, there was an increment

in active time during the remission period compared to the baseline, although this difference did *not* reach significance (255.6±90.6vs.237.8±93.4, respectively;  $p=0.09$ ). On the other hand, non-responders to IFX did not present any significant changes in the active time, inactive time, number of steps taken per day, and distance walked in the SWT (Table 3).

There was a decrease in anxiety and depression scores following IFX-induced remissions compared with the baseline status (Table 2). Nonetheless, the mean depression or anxiety scores *throughout* the study period remained below 8, indicating that subjects presented *psychological scores within a normal range*.

## DISCUSSION

The current study has demonstrated that CD patients that achieve IFX-induced remission present an improvement in PA levels in daily life. To the best of our knowledge, this is the first study that compared objectively measures of daily PA post infliximab intervention, in eligible patients with active CD, to this induction therapy.

It is well known that PA offers many benefits to health even for those who have chronic diseases<sup>4</sup>. The American College of Sports Medicine<sup>20</sup> recommends at least 30 minutes of moderate aerobic activity for five days a week, or 20 minutes of heavy aerobic activity 3-times/week. Tudor-Locke et al.<sup>15</sup> demonstrated that 30 min/day of moderate-to-vigorous PA translated into approximately 7500 steps/day. In our study, IFX responders presented a increase in the number of steps/day i.e., notably nearly 50% of patients reached the mark of 7500 steps. Interestingly, another investigation noticed that an increase in 1000 steps daily was of clinical relevance to people's health<sup>21</sup>. In this context, our research demonstrated that an average increase of 1092 steps occurred in those patients who reached clinical remission.

The PA levels improvement observed after IFX-induced remission in our investigation may define disease activity as responsible for the inability of active CD patients to exercise. In this sense, we observed that patients with IFX refractory-CD have not changed their outcome in terms of active time, inactive time, number of steps taken per day, and distance walked in the SWT. It has also been demonstrated that regular PA is uncommon in IBD patients and about 40%, even though with the disease in remission, have particularly fatigue, joint or abdominal pain as the most common

barriers to exercises. Although most of these symptoms disappear with disease remission, they yet avoid doing it for fear, shame, or insecurity because there are no restrooms near<sup>by</sup><sup>22</sup>. During the last decade, there has been an increase in the number of studies investigating PA in IBD<sup>9,12,23</sup>. However, most of such studies have focused on the evaluation of PA but none have investigated the influence of biological therapy on its behavior. Just one of them has employed an accelerometer in CD patients in remission or in active disease, and no difference was observed between both groups. Moreover, this was a transversal study including a small sample of patients<sup>24</sup>.

It is of clinical importance to highlight that the aim of our investigation was to reach a higher level of PA in active CD patients following IFX-induced clinical remission. *It should be emphasized that* Jones et al. demonstrated that among patients with CD in remission, those with higher exercise levels were significantly less likely to develop active disease at 6 months<sup>23</sup>. Furthermore, an inverse relationship was demonstrated between PA and chronic disease evolution, and higher levels of PA were associated with lower mortality risk, taking into account all causes of death<sup>4,20</sup>.

We also found an increase in the FM index, not accompanied by the LM index in those on clinical remission. Weight loss has been recognized as part of the clinical features of several patients with active CD, although other researchers have described a FM increase after IFX therapy<sup>25</sup>. We believe that this FM increase after disease control may be a consequence of the improvement in quantitative as well as qualitative food intake after biological therapy due to the absence of abdominal symptoms as well as lower catabolism in consequence of better intestinal inflammation control.

Changes in functional capacity estimated by SWT and maximal strength have not been found in the group on clinical remission. Although active CD can lead to sarcopenia and a sedentary lifestyle, it is possible to speculate that the functional capacity of exercising may be preserved in our sample, particularly because it was composed mainly of young adults and with short disease duration. In consequence, the systemic effects that can occur in CD can be light with less impact on functional capacity in this group of patients.

Improvement in PA levels may represent one very important gain induced by biological therapy, besides the benefits previously reported related to symptom remission and mucosal healing. The maintenance of

clinical remission associated with incentives to regular PA may contribute to making that these patients reach an ideal level of PA, according to recommendations, not only to improve life quality but also to reduce the risk of neoplasm and general levels of mortality<sup>3,4,20,23</sup>.

This study presents some limitations: patients with CD were in a tertiary IBD referral center, which tends to make them more likely to have more severe CD than those seen in an outpatient medical care setting. The lack of healthy controls and non-random sample makes it impossible to determine the real impact of IFX treatment on PA levels. Therefore, the hypotheses raised here should be compared with data obtained from future longitudinal studies. Despite these limitations, our findings are useful to show behavior about daily PA habits in patients with CD during disease activity and remission. Furthermore, it should be emphasized that the prospective nature of the present study allowed the systematic and full collection of data, which is an important strength to be considered. Future prospective longitudinal studies are necessary, including a standardized physical activity protocol aimed at patients with remission CD to investigate whether gradually incremented PA results in improved outcomes.

## CONCLUSION

IFX-induced clinical remission followed by maintenance therapy has shown to be effective for increasing PA levels in daily life as well as reduced inactive time on patients with active CD. Given the important role of PA for patients with CD, anti-TNF therapy may be a useful therapeutic strategy along with targeted recommendations for increasing PA levels.

## Conflicts of Interest

J.M.F. Chebli has served as a speaker for Abbott, Abbvie, Janssen, and Takeda. For the remaining authors, there is no conflict to be declared. The authors confirm that this article content presents no conflicts of interest.

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## Author's Contribution

Luca FA: Data curation, investigation, project management, writing - original draft; Malaguti C: Conceptualization, investigation, methodology, supervision, writing - original draft; Chebli LA: Conceptualization, funding, resources, writing - original and final approval; Reboredo MM: Investigation, formal analysis, visualization, writing - original draft; Pinheiro BV: Conceptualization, methodology, funding, resources, writing - original and final approval; Ribeiro TCR:

Data curation, project management, formal analysis, resources; Azevedo FM: Data curation, investigation, formal analysis, interpretation of data; Corrêa JOA: Conceptualization, formal analysis, resources, software, writing - original draft; Gaburri PD: Conceptualization, methodology, visualization, writing - original draft; Chebli JMF: Conceptualization, funding acquisition, project management, resources, supervision, visualization, writing - review & editing.

## RESUMO

**OBJETIVO:** Avaliar a concentração da peptidilarginina deiminase 4 (PAD4) e os polimorfismos de PADI4, como preditores de desenvolvimento de lesão renal aguda, necessidade de terapia renal substitutiva (TRS) e mortalidade em pacientes com choque séptico.

**MÉTODOS:** Foram incluídos indivíduos com idade  $\geq 18$  anos, com diagnóstico de choque séptico na admissão na Unidade de Terapia Intensiva (UTI). Amostras de sangue foram coletadas nas primeiras 24 horas após a admissão do paciente para determinar a concentração sérica de PAD4 e seus polimorfismos PADI4 (rs11203367) e (rs874881). Os pacientes foram acompanhados durante a internação na UTI e tiveram avaliados desenvolvimento da lesão renal aguda séptica (Sepsis-induced acute kidney injury - Saki), necessidade TRS e mortalidade.

**RESULTADOS:** Foram avaliados 99 pacientes; 51,5% desenvolveram Saki e, desses, 21,5% necessitaram de TRS e 80% morreram na UTI. Não houve diferença entre a concentração de PAD4 ( $p=0,116$ ) e seus polimorfismos rs11203367 ( $p=0,910$ ) e rs874881 ( $p=0,769$ ) entre os pacientes. No entanto, o PAD4 apresentou correlação positiva com a concentração plasmática de ureia ( $r=0,269$ ;  $p=0,007$ ) e creatinina ( $r=0,284$ ;  $p=0,004$ ). A concentração de PAD4 e os polimorfismos da PADI4 também não foram associados à TRS e à mortalidade em pacientes com Saki.

**CONCLUSÕES:** A concentração de PAD4 e seus polimorfismos não foram associados ao desenvolvimento de Saki, à necessidade de TRS ou à mortalidade em pacientes com choque séptico. No entanto, as concentrações de PAD4 foram associadas às concentrações de creatinina e ureia nesses pacientes.

**PALAVRAS-CHAVE:** Lesão renal aguda. Proteína-arginina desiminase do tipo 4. Sepsis. Mortalidade. Diálise.

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# The relationship between thyroidectomy complications and body mass index

 Mehmet Üstün<sup>1</sup>  
 Avni Can Karaca<sup>2</sup>  
 İhsan Birol<sup>3</sup>  
 Gülberk Uslu<sup>1</sup>  
 Semra Demirli Atıcı<sup>1</sup>  
 Cengiz Aydın<sup>1</sup>

<sup>1</sup>. University of Health Sciences Tepecik Training and Research Hospital, Department of General Surgery, Izmir, Turkey  
<sup>2</sup>. Izmir University of Economics Faculty of Medicine, Department of General Surgery, Izmir, Turkey  
<sup>3</sup>. 25 Aralık State Hospital Department of General Surgery, Gaziantep, Turkey

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## SUMMARY

**INTRODUCTION:** Obesity is a growing public health problem associated with many comorbid diseases. The aim of this study was to evaluate the relationship between body mass index and complications of thyroidectomy.

**METHODS:** Patients who underwent total thyroidectomy between January 2015 and December 2018 were enrolled. Patients were divided into two groups, i.e., BMI <25 (group A) and BMI ≥ 25 (group B). Demographics, operative time, and complications were retrospectively reviewed.

**RESULTS:** The study included 145 patients (66 in Group A and 79 in Group B). There was no significant difference between the two groups in terms of age ( $p=0.330$ ) and gender ( $p=0.055$ ). No surgical site infection and bleeding complications were observed in any patients. The mean operative time was 148.4 minutes (90–235) in Group A and 153.4 minutes (85–285) in Group B ( $p=0.399$ ). Transient hypocalcemia was observed in 25 (37.9%) patients in group A, and 23 (29.1%) patients in Group B ( $p=0.291$ ). Permanent hypocalcemia was not observed in any patient in group A, and in 2 patients in Group B (2.5%) ( $p=0.501$ ). Transient recurrent nerve palsy was observed in 1 (1.5%) patient in Group A and in 3 (3.8%) patients in Group B ( $p=0.626$ ). None of the patients had permanent recurrent nerve palsy. Parathyroid autotransplantation was performed on 1 patient (1.5%) in group A and on 7 (8.9%) patients in Group B ( $p=0.055$ ).

**CONCLUSION:** We think there is no relationship between a high BMI and thyroidectomy complications, and surgery can also be performed safely in this patient group.

**KEYWORDS:** Thyroidectomy. Thyroid Gland/surgery. Postoperative Complications. Body Mass Index.

## INTRODUCTION

Obesity is a growing public health problem associated with many comorbid diseases. The number of patients with a high body mass index (BMI) undergoing surgical procedures increases with the increasing obesity rate. Considering endocrine surgery, several

studies have reported the relationship between obesity and thyroid cancer or hyperparathyroidism. There are scarce studies reporting the safety and complications of endocrine surgery in high body mass index patients<sup>1-7</sup>. The aim of this study was to evaluate the

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CORRESPONDING AUTHOR: İhsan Birol

Güney Mahallesi 1140/1 Sokak No: 1 Yenisehir Konak İzmir, Türkiye – Sağlık Bilimleri Üniversitesi Tepecik Eğitim ve Araştırma Hastanesi  
Sağlık Bilimleri Üniversitesi Te – İzmir – Turkey – 35180. Tel:+905363624585 – Fax: +902324330756

E-mail: ihsanbirol@gmail.com

relationship between body mass index and thyroidectomy complications including parathyroid autotransplantation rates among other parameters in other studies.

## METHODS

Patients who underwent total thyroidectomy in our center between January 2015 and December 2018 were enrolled in this study. Patients who underwent thyroidectomy for recurrence, hemithyroidectomy, completion thyroidectomy, parathyroidectomy, and lymph node dissection were excluded. Neuromonitoring is not a routine practice in our center due to technical and financial reasons. The cases operated with neuromonitoring were also excluded from the study. All operations were performed by two surgeons. The surgical technique involved conventional standard ligation (clamping-tie) and vascular closure devices for hemostasis. Parathyroid glands were identified and dissected within their capsules maintaining vascularization as much as possible. The recurrent nerve was always identified at the laryngeal penetration point. Parathyroid autotransplantation into the sternocleidomastoid muscle or strap muscles was used as a salvage procedure in case of the devascularization of the glands.

Body mass index (BMI) was calculated using the formula [weight (kg)/height (m<sup>2</sup>)].

Patients were divided into two groups, BMI <25 and BMI ≥ 25. Demographics, American Society of Anesthesiologists (ASA) scores, operation time (minutes), postoperative bleeding complications, parathyroid autotransplantation rates, surgical site infection, postoperative serum calcium level, postoperative recurrent nerve palsy, and length of stay were recorded and retrospectively analyzed.

Hypocalcemia was defined as a postoperative serum calcium level below 8 mg/dl. Hypocalcemia lasting less than 6 months was defined as transient, and lasting more than 6 months was defined as permanent.

Recurrent nerve palsy was diagnosed with indirect laryngoscopy which was performed on patients with dysphonia, dyspnea, and swallowing disorders. Recurrent laryngeal nerve palsy that was persistent for 6 months and documented by laryngoscopy was considered as permanent palsy.

Approval from the institutional research ethics board was obtained (decision number 2019/12-2).

## Statistical Analysis

The 25<sup>th</sup> version of the “Statistics Package for Social Sciences” by International Business Machines Corporation (IBM) (New York, United States) was utilized for statistical analysis. Fisher’s exact t-test was used for comparing discrete variables and one-way ANOVA was used for continuous variables. Logistic regression was used for multivariate analysis. A p-value of less than 0.05 was considered statistically significant.

## RESULTS

A total of 145 patients were enrolled. One hundred twenty-five were female (86.2%) and 20 were male (13.8%). There were 66 patients (45.5%) in the BMI <25 group and 79 patients (54.5%) in the BMI ≥ 25 group. The mean age in the BMI <25 group was 43.9 (17-73) years and the mean age in the BMI ≥ 25 group was 48.2 (24-74) years. In the BMI <25 group, 5 (7.5%) patients were male and 61 (92.5%) were female. In the BMI ≥ 25 group, 15 (19%) patients were male and 64 (81%) were female. There was no statistically significant difference between the two groups in terms of age ( $p=0.330$ ) and gender ( $p=0.055$ ). No surgical site infection and postoperative hematoma/bleeding complications were observed in any patients in both groups.

When both groups were compared in terms of operative time, the mean operative time was 148.4 minutes (90-235) in the BMI <25 group and 153.4 minutes (85-285) in the BMI ≥ 25 group ( $p=0.399$ ).

In the BMI <25 group, 25 (37.9%) patients had transient hypocalcemia postoperatively, whereas in the BMI ≥ 25 group, 23 (29.1%) patients had transient hypocalcemia ( $p = 0.291$ ). Permanent hypocalcemia was not observed in any patient in the BMI <25 group; in the BMI ≥ 25 group, it was observed only in 2 patients (2.5%) ( $p = 0.501$ ). There were no statistically significant differences between the two groups in terms of postoperative transient and permanent hypocalcemia rates.

Transient recurrent nerve palsy was observed in 1 (1.5%) patient in the BMI <25 group and in 3 (3.8%) patients in the BMI ≥ 25 group. None of the patients had permanent recurrent nerve palsy. There was no statistically significant difference between the two groups in terms of postoperative recurrent nerve palsy ( $p=0.626$ ).

In the BMI <25 group, parathyroid autotransplantation was performed on 1 patient (1.5%); in the BMI ≥ 25 group, it was performed on 7 (8.9%) patients. Although

the ratio seems higher in the BMI  $\geq 25$  group, there was no statistically significant difference between the two groups in terms of performing parathyroid autotransplantation ( $p=0.055$ )

No statistically significant difference was found between the two groups in terms of any parameter examined both with univariate and multivariate analyses.

**TABLE 1.** DEMOGRAPHIC AND CLINICAL CHARACTERISTICS OF PATIENTS CLASSIFIED INTO VARIOUS BMI GROUPS

|  | BMI <25<br>group<br>n:66 | BMI $\geq 25$<br>group<br>n:79 | P-value |
|--|--------------------------|--------------------------------|---------|
| Age (Mean)                             | 43.97                    | 48.28                          | 0.330   |
| Gender (n,%)                           |                          |                                |         |
| -Male                                  | 5 (7.6)                  | 15 (19)                        | 0.055   |
| -Female                                | 61 (92.4)                | 64 (81)                        |         |
| ASA Score (n,%)                        |                          |                                |         |
| -ASA1                                  | 7 (10.6)                 | 11 (14)                        | 0.451   |
| -ASA2                                  | 54 (81.8)                | 64 (81)                        |         |
| -ASA3                                  | 5 (7.6)                  | 4 (5)                          |         |
| -ASA4                                  | 0                        | 0                              |         |
| Transient hypocalcemia (n,%)           | 25 (37.9)                | 23 (29.1)                      | 0.291   |
| Permanent hypocalcemia (n,%)           | 0                        | 2 (2.5)                        | 0.501   |
| Transient recurrent nerve palsy (n,%)  | 1 (1.5)                  | 3 (3.8)                        | 0.626   |
| Permanent recurrent nerve palsy (n,%)  | 0                        | 0                              |         |
| Operative time (Mean, minutes)         | 148.4                    | 153.4                          | 0.399   |
| Parathyroid autotransplantation (n, %) | 1 (1.5)                  | 7 (8.9)                        | 0.055   |

## DISCUSSION

The increasing prevalence of obesity also brings an increase in the number of patients with a high body mass index undergoing general surgical procedures. This led us to investigate the relationship between complications of general surgical procedures and a high body mass index.

Thyroidectomy is one of the most common general surgical procedures. Buerba et al.<sup>4</sup> reported an increased rate of wound site complications in obese and morbidly obese patients, and also urinary complications in morbidly obese patients in their study of 26864 patients. The same study showed the relationship between obesity and wound site complications and longer durations of surgery after parathyroidectomy. The authors noted that despite the statistical significance, the difference might not be clinically significant as the complications were rare, they did

not prolong the hospital admission and required reoperation. They concluded that thyroidectomy and parathyroidectomy can be performed safely in this patient group with appropriate surgical decision making. Our study found no wound site infections with a smaller patient group.

Our study found no statistically significant difference between patients with normal and high BMI in terms of wound site infection, hematoma, transient or permanent hypocalcemia, or recurrent nerve injury. Finel et al.<sup>5</sup> reported similar results about complications but prolonged surgery in patients with a high BMI. They attributed this difference to bad exposure due to shorter and wider neck, and harder dissection of the parathyroid tissue in adipose tissue in this patient group.

Milone et al.<sup>6</sup> also reported similar findings and longer duration of operation in patients with BMI  $\geq 25$  in their series of 266 patients with similar comments about anatomy and patient positioning. The harder dissection of the parathyroid tissue in the adipose tissue can also result in a devascularization of the parathyroid glands. We, therefore, included the need for parathyroid autotransplantation to our variables and found a higher rate (1.5% vs 8.9%) in patients with a high BMI. However, the difference was not statistically significant and did not result in a longer operative time.

Hypocalcemia, either temporary or permanent, is a common and important problem after thyroidectomy. Current meta-analyses report a rate of 19%-38% transient and 0%-3% permanent hypocalcemia after thyroidectomy<sup>8</sup>. Our study revealed similar rates in both groups with no increase in patients with a high BMI.

Recurrent nerve palsy is one of the most serious complications of thyroidectomy. Current literature reveals a rate of 9.8% (1.4%-38.4%) transient and 2.3% (0%-18.6%) permanent recurrent nerve palsy after thyroidectomy<sup>9</sup>. Our study found similar rates for temporary injury and no cases of permanent ones. The latest paper about obesity and thyroidectomy was published in 2019 by Farag et al.<sup>7</sup> and it also reports no relationship between obesity and thyroidectomy complications. Our study found similar results with previous reports. The retrospective design and number of patients are limitations of our study.

## CONCLUSION

We think there is no relationship between a high BMI and thyroidectomy complications, and surgery can also be performed safely in this patient group.



Multicenter prospective studies and metanalyses can increase knowledge in the subject.

### Financial Disclosure

The authors declare that this study has received no financial support.

### Conflicts of Interest

No conflict of interest was declared by any of the authors.

### Author's Contribution

MU and IB designed and prepared the study. MU wrote the article. SDA and GU collected the patients' data. IB and ACK helped draft the study. IB, ACK, MU, GU, and SDA participated in the surgical and medical treatment of the patients. CA designed the study, participated in the design and coordination, and helped draft the study. All authors have read and approved the last version of the article.

## RESUMO

**INTRODUÇÃO:** A obesidade é um crescente problema de saúde pública associado a muitas doenças comórbidas. O objetivo deste estudo foi avaliar a relação entre o índice de massa corporal e as complicações da tireoidectomia.

**MÉTODOS:** Os pacientes submetidos à tireoidectomia total entre janeiro de 2015 e dezembro de 2018 foram incluídos. Os pacientes foram divididos em dois como IMC <25 (grupo A) e IMC ≥ 25 (grupo B). Demografia, tempo operatório e complicações revisadas retrospectivamente.

**RESULTADOS:** O estudo incluiu 145 pacientes (66 no grupo A e 79 no grupo B). Não houve diferença significativa entre os dois grupos em termos de idade ( $p = 0,033$ ) e sexo ( $p = 0,055$ ). Nenhuma infecção do sítio cirúrgico e complicações hemorrágicas foram observadas em nenhum paciente. O tempo operatório médio foi de 148,4 minutos (90-235) no grupo A e 153,4 minutos (85-285) no grupo B ( $p = 0,399$ ). Hipocalcemia transitória foi observada em 25 (37,9%) pacientes do grupo A e 23 (29,1%) do grupo B ( $p = 0,291$ ). Hipocalcemia permanente não foi observada em nenhum paciente do grupo A e em 2 pacientes do grupo B (2,5%) ( $p = 0,501$ ). Paralisia nervosa recorrente transitória foi observada em 1 (1,5%) paciente no grupo A e em 3 (3,8%) pacientes no grupo B ( $p = 0,626$ ). Nenhum dos pacientes apresentou paralisia nervosa recorrente permanente. O autotransplante de paratireóide foi realizado em 1 paciente (1,5%) no grupo A e em 7 (8,9%) pacientes no grupo B ( $p = 0,055$ ).

**CONCLUSÃO:** Acreditamos que não há relação entre um IMC alto e as complicações da tireoidectomia e a cirurgia pode ser realizada com segurança também neste grupo de pacientes.

**PALAVRAS-CHAVE:** Tireoidectomia. Glândula Tireoide/cirurgia. Complicações Pós-Operatórias. Índice de Massa Corporal.

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# Correlation between hepatopulmonary syndrome and oxygen saturation pulse oximetry in cirrhotic patients

 Sara Cardoso Paes Rose<sup>1</sup>  
 Débora Vilela Cunha<sup>1</sup>  
 Sandra de Barros Cobra Medeiros<sup>3</sup>  
 José Eduardo Trevizoli<sup>2</sup>  
 Marcos de Vasconcelos Carneiro<sup>2</sup>  
 Wladimir Magalhães de Freitas<sup>2</sup>  
 Liliana Sampaio Costa Mendes<sup>2</sup>

<sup>1</sup>. Faculdade de Medicina, Universidade Católica de Brasília, Brasília, DF, Brasil  
<sup>2</sup>. Serviço de Gastroenterologia, Instituto Hospital de Base do Distrito Federal, Brasília, DF, Brasil  
<sup>3</sup>. Serviço de Cardiologia, Instituto Hospital de Base do Distrito Federal, Brasília, DF, Brasil

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## SUMMARY

Hepatopulmonary Syndrome (HPS) is a complication of cirrhosis that worsens the disease's prognosis, pre and post liver transplant. The objective of this study is to analyze the prevalence of HPS in cirrhotic patients at our service and to correlate it with oxygen saturation (SatO<sub>2</sub>) using a pulse oximeter to evaluate if this is useful as a screening test for HPS. A prospective study was conducted in patients with hepatic cirrhosis conventionally selected from 2014 to 2016. All the patients underwent an echocardiogram with microbubbles and oxygen saturation measurement by pulse oximetry. Those with intrapulmonary shunt were submitted to arterial blood gas analysis. The relationship between oxygen saturation and HPS was assessed by the multivariate model of binary logistic regression. We analyzed 77 patients, and 23.3% (18 patients) had all criteria for HPS. The relationship between HPS and SatO<sub>2</sub> did not show statistical significance, even after the variables were adjusted for sex, age, and smoking. Oxygen saturation alone was not able to detect HPS in the sample of cirrhotic patients. More accurate methods for screening and diagnosis of the syndrome should be used.

**KEYWORDS:** Hepatopulmonary syndrome. Oximetry. Triage.

## INTRODUCTION

Hepatopulmonary syndrome (HPS) is defined as systemic arterial hypoxemia due to vasodilatations of intrapulmonary capillaries in the presence of liver disease. It is a complication of cirrhosis, with a prevalence of 4-32% in this population<sup>1-3</sup>. Its pathophysiology is not fully understood and current knowledge on the subject is based mainly on animal experiments and data from patients who are candidates for liver transplantation<sup>1</sup>generally at a cirrhotic stage. Knowledge about the subject is still only partial. The majority of

the information about the etiopathogenesis of HPS has been obtained through experiments on animals. Reported prevalence in patients who are candidates for a liver transplantation (LT).

The pathogenesis of HPS occurs due to the vasodilation of intrapulmonary capillaries. The presence of pulmonary arteriovenous communications (shunts) allows oxygenated blood to mix with non-oxygenated blood, leading to hypoxemia<sup>2-4</sup>occurring in 10-30% of patients with cirrhosis. It is due to vasodilation and

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CORRESPONDING AUTHOR: Sara Cardoso Paes Rose

Endereço: SQSW 102 bl. E apt 401 – Brasília, DF – Brasil (CEP 70670205) – Tel: 55 61 992289129

Email: saracprose@gmail.com

angiogenesis in the pulmonary vascular bed, which leads to ventilation-perfusion mismatching, diffusion limitation to oxygen exchange, and arteriovenous shunting. There is evidence, primarily from animal studies, that vasodilation is mediated by a number of endogenous vasoactive molecules, including endothelin-1 and nitric oxide (NO). The diagnosis of HPS is obtained by detecting the presence of intrapulmonary shunt and hypoxemia in a patient with liver disease<sup>1,5-6</sup> generally at a cirrhotic stage. Knowledge about the subject is still only partial. The majority of the information about the etiopathogenesis of HPS has been obtained through experiments on animals. Reported prevalence in patients who are candidates for a liver transplantation (LT). Even though most patients are asymptomatic, HPS decreases the quality of life and worsens the prognosis of these patients, regardless of the degree of liver disease.<sup>7,8</sup> The diagnosis of HPS and oxygen saturation. Thirty cirrhotic patients considered for liver transplantation were enrolled in this study. Pulse oximetry and lung perfusion scintigraphy were conducted in patients while they were in supine and upright body positions. Pathological IPBS was observed in 16% and 20% of patients examined in the supine and upright body positions, respectively (mean difference 0.59%;  $P = 0.046$ ).

The only effective treatment for HPS is liver transplantation<sup>9</sup>. After this procedure, an important improvement of symptoms may occur, and it is possible to completely reverse the condition. However, HPS is also a factor that worsens the prognosis after transplantation, especially in those with PaO<sub>2</sub> less than 50 mmHg, when compared to those without the syndrome.<sup>10,11</sup> Three pulmonary complications specific to chronic liver disease have been characterized: hepatopulmonary syndrome (HPS). The presence of HPS with PaO<sub>2</sub> less than 60 mmHg is an independent criterion for priority liver transplantation, despite the value of the MELD score, according to the current regulation of liver transplantation in Brazil<sup>12</sup>.

Therefore, an effective method for screening HPS is highly necessary<sup>13</sup>. There is no well-established protocol for verifying the presence of shunt and HPS in pre-transplant patients. Several transplant centers in the country and in the world do not perform an exam for detecting the presence of intrapulmonary shunt as a routine before transplantation. Many centers use oxygen saturation by pulse oximetry (SatO<sub>2</sub>) for SHP screening<sup>1,14</sup> generally at a cirrhotic stage. Knowledge about the subject is still only partial. The majority of

the information about the etiopathogenesis of HPS has been obtained through experiments on animals. Reported prevalence in patients who are candidates for a liver transplantation (LT).

The objective of this study is to analyze the prevalence of HPS in cirrhotic patients in our service and correlate it with oxygen saturation (SatO<sub>2</sub>) by pulse oximeter to verify if this could be a reliable screening test for the diagnosis of HPS.

## METHODS

This was a prospective study that included patients with liver cirrhosis who attended the hepatology outpatient clinic at the Hospital de Base do Distrito Federal from 2014 to 2016. The diagnosis of cirrhosis in these patients was previously made through a histopathological study or by a combination of clinical, radiological and laboratory findings. Patients with hepatocarcinoma, active infectious process, hospitalization in the previous 30 days, and those who underwent liver transplantation were excluded from the series.

Oxygen saturation by pulse oximetry (SatO<sub>2</sub>) was measured for all patients during their participation in the study. The SatO<sub>2</sub> value of 96% was considered as the cutoff point for analysis. An echocardiogram with microbubbles was also requested for all patients to verify the presence or absence of intrapulmonary shunt. All those who had a positive intrapulmonary shunt were subjected to arterial blood gas analysis to measure the alveolar-arterial gradient and PaO<sub>2</sub>. All patients underwent chest X-ray and spirometry, and those who presented abnormalities in these exams were excluded.

The criteria for hepatopulmonary syndrome in our study were the presence of liver disease with positive intrapulmonary shunt and hypoxemia, PaO<sub>2</sub> <80 mmHg, and/or alveolar-arterial gradient greater than 15 mmHg or greater than 20 mmHg, in patients older than 64 years<sup>15</sup>. Patients with HPS were classified as mild (PaO<sub>2</sub> > 80 mmHg), moderate (PaO<sub>2</sub> between 60 and 80 mmHg), and severe (PaO<sub>2</sub> ≤ 60 mmHg).

Continuous variables with normal and non-parametric distribution were evaluated by mean, standard deviation, median, and interquartile range. Nominal percentages and ordinal variables were analyzed by median and interquartile range. The chi-square test was used to compare proportions, when necessary.

For the comparison of continuous or orderly measurements between two groups, the Mann-Whitney test was applied. The relationship between SatO<sub>2</sub> and HPS was evaluated by the multivariate model of binary logistic regression. The individuals were characterized in the dependent variable “presence” or “absence” of HPS first in an unadjusted model and later adjusted for the variables of sex, age and smoking.

All patients enrolled in the study consented to their participation through the Informed Consent Form. This study was approved by the Brazilian Ethics Committee (Pesquisa/Fepecs/Plataforma Brasil - CAAE: 47514315.7.0000.5553V2).

## RESULTS

In the beginning, the study sample included 102 patients. There was a loss of 25 patients (24.5% of total). The main reasons for this were: refusal to perform the requested tests and giving up on continuing the study (13 patients, 12, 7%). Four patients (3.9%) died in the period. One patient (0.9%) was excluded after liver transplantation. In the end, 77 patients (75.4%) were evaluated.

The characteristics of the sample can be seen in Table 1. Liver cirrhosis due to C virus was the most prevalent (33.7%, 26 patients), followed by alcoholic cirrhosis (24.6%, 19 patients). Cirrhosis classified as Child-Pugh A was the most prevalent (68%, 52 patients). Patients with Child-Pugh B cirrhosis and

**TABLE 1.**

|                           |               |
|---------------------------|---------------|
| Gender: Male (%. n)       | 51.9%. 41     |
| Age. years                | 54.6 +/- 10.6 |
| BMI. kg/m <sup>2</sup>    | 26 +/- 4.7    |
| SatO <sub>2</sub> (%)     | 97% (95-97)   |
| Current smoking (%. n)    | 13.9%. 11     |
| Previous smoking (%. n)   | 26.6%. 26.5   |
| Current alcoholism (%. n) | 6.3%. 5       |
| Previous alcoholism(%..n) | 26.6%. 21     |

Child-Pugh C cirrhosis were 26% (20 patients) and 6% (5 patients), respectively.

The patients that had positive intrapulmonary shunt were 77, 29.8% (23 patients), however, only 23.3% (18 patients) had all the criteria for HPS. Among the patients with HPS 55.5% (10 patients) had the syndrome in its mild form, 38.8% (7 patients) had a moderate form and 5.5% (1 patient) had a severe form of HPS (Figure 1).

There were no patients with mild HPS that had SatO<sub>2</sub> less than 96%. Among those with the moderate syndrome, 42.8% (3 patients) had a SatO<sub>2</sub> less than 96%, and 57.2% (4 patients) had a SatO<sub>2</sub> greater than or equal to 96%. Only one patient had severe HPS and a SatO<sub>2</sub> equal to 98%. In a multivariate model of logistic regression, the determination of SatO<sub>2</sub> alone was not able to predict individuals with HPS even after adjusting for age, sex, and current and previous smoking. The relationship between HPS and SatO<sub>2</sub>, using the binary logistic regression model, showed an unadjusted exponent B value of 1.12 (IC 0.77 - 1.6, p =

**FIGURE 1.**

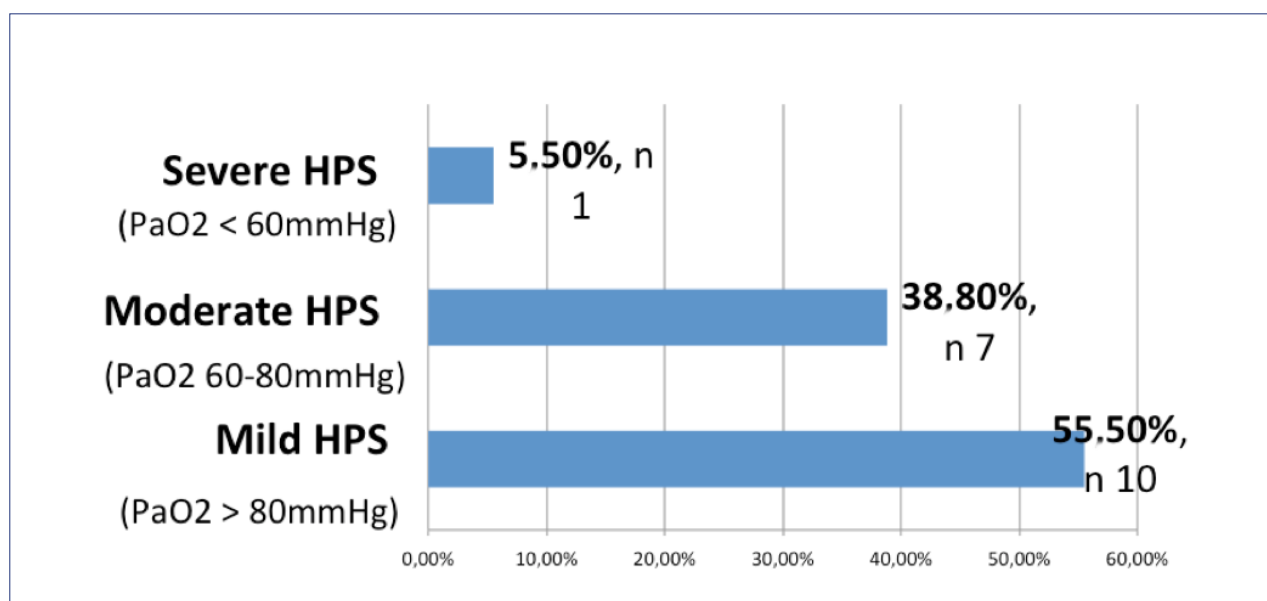


TABLE 2.

| Variable                    | Ex-<br>p(B) | p     | C.I of 95% for<br>EXP (B) |          |
|-----------------------------|-------------|-------|---------------------------|----------|
|                             |             |       | Inferior                  | Superior |
| SatO2                       | 1.127       | 0.526 | 0                         | 1.628    |
| SatO2 + Age                 | 0.965       | 0.235 | 0.910                     | 1.023    |
| SatO2 + Age + Sex           | 1.355       | 0.618 | 0.411                     | 4.469    |
| SatO2 + Age + Sex + Smoking | 0.835       | 0.836 | 0.152                     | 4.577    |

0.52). After the variables were adjusted for sex, age, and current and past smoking, the adjusted B exponent value was 0.83 (IC 0.15 - 4.5,  $p = 0.83$ ). In both cases, there was no statistical significance (Table 2).

## DISCUSSION

The evaluation of oxygen saturation by pulse oximetry is a non-invasive, low-cost method, and, for this reason, it has been used as screening for HPS. The cut-off values for HPS have not yet been well established. The most used value was proposed by Arguedas et al, in 2007, after a study with 127 patients referred for liver transplant evaluation. Of these, 40 patients had HPS. The oxygen saturation value by pulse oximetry below 96% obtained sensitivity and specificity, respectively, of 100% and 88% in detecting patients with PaO<sub>2</sub> below 60 mmHg<sup>16</sup>.

Kochar et al.<sup>17</sup> proposed that patients with HPS evolve with a significant decrease in SatO<sub>2</sub>, when compared with those without HPS. He conducted a 20-month prospective cohort that compared 22 patients with HPS with 32 patients without the syndrome.

However, in our study, the relationship between oxygen saturation by pulse oximetry and HPS through logistic regression obtained a non-significant p-value and an unreliable confidence interval (CI). This indicates that SatO<sub>2</sub>, as an isolated method, was not able to distinguish patients with HPS from those without the syndrome.

The prevalence of HPS was 23.3% in our study, similar to what is found in the literature<sup>13</sup> generally at a cirrhotic stage. Knowledge about the subject is still only partial. The majority of the information about the etiopathogenesis of HPS has been obtained through experiments on animals. Reported prevalence in patients who are candidates for a liver transplantation (LT. However, there was a higher prevalence of mild to moderate HPS (94.3%), when compared to the values found in the literature (77-88%)<sup>1</sup> generally at a cirrhotic

stage. Knowledge about the subject is still only partial. The majority of the information about the etiopathogenesis of HPS has been obtained through experiments on animals. Reported prevalence in patients who are candidates for a liver transplantation (LT. The results in our sample may be a reason why SatO<sub>2</sub> has not been an effective method for screening for HPS, as it can detect more severe cases of the syndrome<sup>16</sup>. However, lower levels of SatO<sub>2</sub> was not observed in moderate to severe cases of HPS in our sample, as was reported by Kochar et al.<sup>17</sup>. Most patients with moderate HPS presented SatO<sub>2</sub> greater than or equal to 96% (57.2%), and one patient with severe SHP had normal SatO<sub>2</sub> (98%).

Hoerning et al.<sup>18</sup> conducted a study with 57 children aged from 1 to 17 years (mean of 4.6 +/- 5.0 years) with liver cirrhosis to verify the usefulness of pulse oximetry in patients with HPS. Despite being a study performed in children, pulse oximetry was also not a useful method for the early diagnosis of HPS, but only for more severe cases of HPS. A total of 18 patients with intrapulmonary shunt had SatO<sub>2</sub> above 98%. Of these, 2 patients (11%) had moderate HPS, with P (A-a) O<sub>2</sub> > 15mmHg and PaO<sub>2</sub> < 70mmHg, despite normal saturation, and died before obtaining a liver transplant<sup>18</sup> the importance of pulse oximetry in diagnosing HPS, and the longitudinal course after liver transplantation in children with cirrhosis referred for liver transplantation. Study design Fifty-six patients aged 1-17 years (mean age, 4.6 ± 5.0 years).

Pulse oximetry can also have other limitations when used as a screening test for all cirrhotic patients. It may not be precise enough when compared to arterial blood gases (PaO<sub>2</sub>) and tends to overestimate the values of arterial oxygenation, especially in smoking individuals<sup>119</sup> generally at a cirrhotic stage. Knowledge about the subject is still only partial. The majority of the information about the etiopathogenesis of HPS has been obtained through experiments on animals. Reported prevalence in patients who are candidates for a liver transplantation (LT. This should be considered, particularly when the number of smoking patients in the sample is important. In our sample, 13.9% (11 patients) were smokers during the study period and 26.6% were former smokers.

The limitations of our study were the reduced number of patients, because of the losses due to non-follow-up. Prospective studies that assess the relationship between SatO<sub>2</sub> and HPS with a higher number of patients are still quite scarce.



Thus, oxygen saturation was not a good screening test for HPS in cirrhotic patients in our study. The higher prevalence of patients with mild HPS in our sample may have had a considerable impact on these results. Performing an echocardiogram with microbubbles in all cirrhotic patients, especially those in waiting for a transplant can be a useful method to detect patients with HPS. The earlier detection of this syndrome can lead to a better evaluation of patients and to further knowledge of its natural history. It can also lead to indications of liver transplant at an earlier and more opportune moment, before other complications of cirrhosis may limit the results of this operation.

## CONCLUSION

Oxygen saturation measured by pulse oximetry, as an isolated method, was not able to screen HPS among a sample of cirrhotic patients. Lower levels of saturation may indicate the presence of HPS; however, the absence of this finding does not exclude the presence of the syndrome. Thus, the use of more accurate tests, such as the echocardiogram with microbubbles associated with arterial blood gases,

is a more accurate alternative for screening and diagnosing HPS, especially in patients in the liver transplant queue.

## Conflicts of interest

There were no conflicts of interest between the authors of this study.

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Biocardios

## Author's Contribution

SCPR contributed with project management, data curation, formal analysis, investigation, methodology, writing of the original draft, review and editing. DVC contributed with data curation and formal analysis. SBC contributed with data curation and investigation. JET contributed with conceptualization, project management, and resources. MVC contributed with conceptualization, methodology, resources, review and editing. WMF contributed with formal analysis, software, and validation. LSCM lead the project management and supervision and contributed with the conceptualization, investigation, methodology, resources, validation, review and editing.

## RESUMO

A Síndrome Hepatopulmonar (SHP) é uma complicação da cirrose que piora o prognóstico da doença pré e pós-transplante hepático. O objetivo do trabalho é analisar a prevalência de SHP em pacientes cirróticos de nosso serviço e correlacioná-la com a saturação de oxigênio (SatO2) pelo oxímetro de pulso, e avaliar se este seria útil como um exame de triagem no diagnóstico de SHP. Foi realizado um estudo prospectivo em pacientes portadores de cirrose hepática no período de 2014 a 2016. Todos os pacientes foram submetidos a um ecocardiograma com microbolhas e a saturação de oxigênio pela oximetria de pulso. Aqueles com shunt intrapulmonar foram submetidos a gasometria arterial. A relação entre a saturação de oxigênio e SHP foi avaliada pelo modelo multivariado de regressão logística binário. Foram analisados 77 pacientes, destes 23,3% (18 pacientes) apresentaram todos os critérios para SHP. A relação entre a SHP com a SatO2 não obteve significância estatística, mesmo após as variáveis terem sido ajustadas pelo sexo, idade e tabagismo atual ou passado. A saturação de oxigênio, de forma isolada, não foi capaz de distinguir a SHP na amostra de pacientes cirróticos em nosso estudo. Deve-se utilizar métodos mais acurados para a triagem e diagnóstico dessa síndrome.

**PALAVRAS-CHAVE:** Síndrome hepatopulmonar. Oximetria. Triagem.

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# A comparison of IL-17 and IL-34 concentrations in the cerebrospinal fluid of patients with acute inflammatory demyelinating neuropathy and chronic inflammatory demyelinating polyneuropathy

 Hayriye Soytürk<sup>1</sup>  
 Murat Yılmaz<sup>2</sup>

<sup>1</sup>. Bolu Abant İzzet Baysal University, Gölköy /Bolu, Turkey

<sup>2</sup>. Bolu Abant İzzet Baysal University, Faculty Of Medical School, Department of Neurology Gölköy/Bolu, Turkey

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## SUMMARY

**OBJECTIVE:** The role of interleukins, such as IL-17 and IL-34, in the pathogenesis of autoimmune diseases has been established in the literature. In the current study, we aimed to identify the concentrations of IL-17 (IL-17A, IL-17F) and IL-34 in the cerebrospinal fluid (CSF) of patients with chronic inflammatory demyelinating polyneuropathy (CIDP) and acute inflammatory demyelinating neuropathy (AIDN).

**METHODS:** We included in this study 8 patients with CIDP (none of them receiving immunomodulatory or immunosuppressant therapy), 7 patients with Guillain-Barre syndrome (GBS, AIDN), and 7 control subjects. The CIDP and AIDN diagnoses were made by clinical evaluation and electrophysiological investigations according to international criteria. CSF samples were obtained appropriately, and the levels of IL-17A, IL-17F, and IL-34 were measured by ELISA kits.

**RESULTS:** The concentrations of IL-17A, IL-17F, and IL-34 were higher in those with CIDP and AIDN compared to the controls ( $p=0.005$ ,  $p=0.01$ , and  $p=0.001$ , respectively). While IL-34 levels were significantly higher in AIDN patients than in CIDP patients ( $p=0.04$ ), there were no significant differences between the AIDN and CIDP groups with regard to the levels of IL-17A and IL-17F ( $p=0.4$  and  $p=0.2$ , respectively).

**CONCLUSION:** Our results indicate that IL-17A, IL-17F, and IL-34 levels may have a role in CIDP and AIDN. Furthermore, the difference in the IL-34 levels of patients with AIDN and CIDP may indicate an important difference between the pathogenesis of these two sets of the disease.

**KEYWORDS:** Gullian-Barre Syndrome. Cytokines. Interleucinas. Interleukin-17. Interleukin-34.

## INTRODUCTION

GBS is a post-infectious autoimmune disease that causes symmetrical motor weakness and sensorial loss. It is the second leading cause of acute and sub-acute generalized paralysis. Electrophysiologically speaking, there are three subtypes of this syndrome: acute motor axonal neuropathy (AMAN), which has

the worst prognosis; acute motor-sensory axonal neuropathy (AMSAN); and acute inflammatory demyelinating neuropathy (AIDN), which is mostly known as the classical form of GBS<sup>1</sup>. The pathophysiology of AIDN is characterized by the segmental demyelination of the proximal myelinated neurons in roots and

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CORRESPONDING AUTHOR: Hayriye Soytürk

Bolu Abant İzzet Baysal University, Gölköy /Bolu/ Turkey – Tel.: +90 5549338864

Email: hayriyesoyturk1@gmail.com

plexuses. Despite the fact that the underlying immunological mechanism is yet to be fully understood, current evidence shows that both humoral and cellular pathways are involved in the pathogenesis. There is some evidence in the literature that T cells play a crucial role in the AIDN pathophysiology. For instance, T lymphocytes and macrophages have been shown to infiltrate peripheral nerves and directly attack myelin proteins P0, P2, and PMP22<sup>2</sup>. Furthermore, in addition to the increase in cerebrospinal fluid (CSF) concentrations of T-cell-associated cytokines (such as IL-17, IFN- $\gamma$ , and IL-22), the blood levels of T helper (Th) 1 and Th-17 have been found to be increased in AIDN<sup>3,4</sup>. Recent studies have also shown that IL-14, IL-12, IL-23, IL-27, IL-35 also partake in the immune response<sup>5</sup>. However, the role of other cytokines (such as IL-34) in the pathogenesis of AIDN is unknown.

Chronic inflammatory demyelinating polyneuropathy (CIDP) is another similar immune-mediated autoimmune disease. Its clinical findings are similar to those of AIDN; however, the disease course in this condition demonstrates a chronic relapsing-remitting characteristic<sup>6</sup>. The pathophysiology of CIDP is also unclear. In many studies, the presence of both humoral and cell-mediated immune responses have been reported<sup>7,8</sup>. Presumably, as is the case in AIDN, the main target for these immune cells is the myelin. Matsumuro et al.<sup>9</sup> and Rizutta et al.<sup>10</sup> found that CD4<sup>+</sup> and CD8<sup>+</sup> T cells activate macrophages in concert, which then infiltrate the sural nerve. They concluded that T cells, therefore interleukins, may have a critical role in CIDP development<sup>9,10</sup>.

IL-17 is a pro-inflammatory cytokine that consists of 150 amino acids. It has 6 subtypes, all of which share sequence homology but are distinct with regard to tissue expression. They are produced by Th17 and their overexpression causes several autoimmune diseases including experimental autoimmune encephalomyelitis (EAE), a murine model of multiple sclerosis (MS), and rheumatoid arthritis<sup>11</sup>. In recent studies, there have been conflicting results about the association between IL-17 and CIDP, while most studies have reported that IL-17 may have an important role in CIDP<sup>12-14</sup>, some researchers did not find any association<sup>15</sup>. On the other hand, the role of IL-17 in AIDN is relatively clearer, as most of the studies have determined a critical role for IL-17 in AIDN pathophysiology<sup>16,17</sup>.

IL-34 is a hematopoietic cytokine that plays a key role in the survival, proliferation, and differentiation

of myeloid stem cells<sup>18</sup>. Particularly, IL-34 is suggested to have an important role in the preservation of central nervous system homeostasis by its action(s) on different cell types including neurons, microglia, and endothelial cells. In the latest studies, there is growing evidence that IL-34 contributes to the pathogenesis of various conditions including autoimmune disorders<sup>19</sup>.

In this study, we aimed to identify the concentrations of IL-17 (IL-17A, IL-17F) and IL-34 in CSF samples from patients with CIDP and AIDN.

## METHODS

This study was conducted in the Abant İzzet Baysal University Medical Faculty, Department of Neurology, from 2013 to 2017. The study and its conduct were approved by the local ethics committee (protocol number; 2018/203) and informed consent forms were obtained from all participants. The Helsinki Declaration was followed during the study period and the writing of the article.

We included in this study a total of 8 CIDP, 7 GBS (AIDN) patients, and 7 control subjects. CIDP patients met the Possible, Probable, or Definite criteria for Typical or Atypical CIDP of the EFNS/PNS Joint Task Force<sup>20</sup>. None of the patients with CIDP were using any medication for CIDP such as immunomodulatory or immunosuppressant drugs at the time of CSF withdrawal. GBS was diagnosed according to the international criteria for GBS or its variants, and AIDN diagnosis was defined and confirmed via electrophysiological investigations<sup>21</sup>. Both CIDP and AIDN patients did not have any accompanying diseases which could confound any measurements (such as other autoimmune diseases, Systemic Lupus Erythematosus, Sjögren syndrome, and diabetes mellitus) performed in this study. The patients constituting the control group were patients with normal pressure hydrocephalus and no additional pathology was detected after CSF results. All participants' sociodemographic and clinical data were recorded.

Electrophysiological examinations were performed via EMG device in the same room with a temperature of 24-25 °C, by the same investigator for all patients. All patients underwent the tests in a quiet room, lying on the examination desk in the supine position. The control group was comprised of patients who did not have a history of neurological or psychiatric disease and had been suspected to have normal pressure hydrocephalus (NBH), but evaluations showed that

they did not have NBH or any other neurological disease. All controls' detailed neurological examinations were completely normal. CSF samples were obtained via appropriate methods from all participants and samples were stored at -80 °C until measurements were performed. In both groups, the levels of IL-17A, IL-17F, and IL-34 levels (pg/mL) were measured from the CSF samples via commercially available ELISA kits (Elabscience and Cloud-Clone, USA). The biochemical parameters of the CSF were measured.

### Statistical analysis

Data were analyzed using SPSS IBM 20.0 (SPSS Inc., Chicago IL) statistics software. The distribution of quantitative variables was assessed using the Kolmogorov-Smirnov test and results were presented as median (25<sup>th</sup> percentile- 75<sup>th</sup> percentile). Categorical data were given as frequency and percent values (%). The comparison of categorical variables was performed using chi-squared tests (Pearson and Fisher's exact tests), while quantitative variables were compared using the Kruskal Wallis tests for 3-group comparisons, while the Mann-Whitney U test was used for 2-group comparisons. The level of statistical significance was set at  $p < 0.05$ .

### RESULTS

All three groups were similar in terms of age and gender ( $p=0.253$  and  $p=0.959$  respectively). The average age was 60.8 in the control group, 64.8 in the CIDP group, and 68.0 in the AIDN group. The gender distribution was 2F/5M in AIDN, 1F/7M in CIDP, and 2F/5M in subjects in the control group. All patients with AIDN

were found to have a history of infection prior to the development of GBS.

There were significant differences between groups in terms of IL-17A, IL-17F, and IL-34 concentrations; these interleukin levels were higher in those with CIDP and AIDN compared to controls ( $p=0.005$ ,  $p=0.01$ , and  $p=0.001$ , respectively). While IL-34 levels were significantly higher in the AIDN group than in the CIDP group ( $p=0.04$ ), there were no significant differences between the AIDN and CIDP groups regarding IL-17A and IL-17F ( $p=0.4$  and  $p=0.2$  respectively). CSF protein and albumin levels were significantly higher in CIDP and AIDN groups compared to controls ( $p=0.023$ ), but there were no significant differences between the CIDP and AIDN groups ( $p=0.23$ ). Other biochemical CSF findings (glucose, Na, Cl, LDH) were similar in all three groups (Table 1).

### DISCUSSION

In the current study, the CSF concentrations of IL-17A, IL-17F, and IL-34 in patients with AIDN and CIDP were found to be significantly higher than in controls, and there were no differences between the CSF findings of the AIDN and CIDP groups regarding IL-17A and IL-17F levels. Apart from these results, the most interesting finding of this study can be seen as the higher levels of IL-34 in patients with AIDN compared to those with CIDP<sup>21</sup>.

Although it may be considered that there is an ongoing debate about the function(s) of IL-34, the majority of recent studies have found that it takes part in the differentiation and survival of macrophages, monocytes, and dendritic cells in response

**TABLE1.** CEREBROSPINAL FLUID FINDING OF THREE GROUPS

|                     | AIDN (n=7)     | CIDP (n=8)    | Control (n=7)  | p   |
|---------------------|----------------|---------------|----------------|---|
| CSF IL-17A (pg/ml)  | 48 (37-96)     | 35 (35-51)    | 6 (6-8)        | $p=0.005$   |
| CSF IL-17F (pg/ml)  | 21 (9-89)      | 8 (2-14)      | 2 (2-3)        | $p=0.010$   |
| CSF IL-34 (pg/ml)   | 57 (38-65)     | 22 (19-44)    | 3 (3-7)        | $p=0.001$   |
| CSF protein (mg/dL) | 71 (71-105)    | 96(75-172)    | 23(23-36)      | $p=0,027$   |
| CSF Albumin (mg/dL) | 6.06 (4.5-6.7) | 5.88(5-7)     | 2.45 (2.1-2.6) | $p=0.013$   |
| CSF Na (mEq/l)      | 135 (135-137)  | 133 (131-134) | 136 (132-139)  | $p=0.578$   |
| CSF Cl (mEq/l)      | 2              |               |                | .8 (2.8-3.2)<br>2.7 (2.7-3)<br>2.6 (2.6-3.2)<br>$p=0.760$ |
| CSF LDH (mg/dL)     | 68 (64-75)     | 57 (46-67)    | 40 (40-51)     | $p=0.662$   |
| CSF Glucose (mg/dL) | 42 (41-62)     | 33(33-42)     | 29(29-31)      | $p=0.428$   |

AIDN: acute inflammatory demyelinating neuropathy; CIDP: chronic inflammatory demyelinating neuropathy; CSF: cerebrospinal fluid; IL: interleukin; LDH: lactate dehydrogenase. Data were presented as median (25<sup>th</sup> percentile- 75<sup>th</sup> percentile)



to inflammation<sup>22</sup>. In the nervous system, cytokines are primarily produced by the neurons and present important effects in the central nervous system (CNS); it has been shown to activate the p-CREB pathway by binding Colony Stimulating Factor 1 Receptors (CSF R1) and upregulates factors that are involved in maintaining protection against neurotoxins<sup>23</sup>. In 2011, Mizuno et al.<sup>24</sup> found that IL-34 provides microglial proliferation and up-regulates Insulin-Degrading Enzyme (IDE) and Heme Oxygenase-1 (HO-1), which is suggested to be the mechanism by which it provides a neuroprotective effect against Oligomeric Amyloid- $\beta$  neurotoxicity. In 2012, a study by D. Ma et al.<sup>25</sup> supported this theory.

When the literature was reviewed, we did not find any study evaluating IL-34 concentrations in neither CIDP nor AIDN. Due to the elevated IL-34 concentrations in the CSF of both CIDP and AIDN in our study, we believe that IL-34 may have an important role in the immune activation of patients with CIDP and AIDN. Furthermore, the difference between IL-34 levels in patients with AIDN and CIDP may suggest the presence of an important difference between these clinically and characteristically similar diseases. It is well known that some AIDN cases can evolve into CIDP with time (in other words some CIDP cases may present with AIDN); however, it is currently impossible to determine whether CIDP will occur. Thus, the levels of IL-34 or the causes/results of its increase may be utilized as a marker to differentiate between these diseases and also to estimate whether a case with AIDN can evolve into CIDP. However, we are aware that our results require confirmation, and further studies with prospective design and adequate follow-up of patients must be performed to determine whether IL-34 levels can be utilized in this manner. It is rather apparent that this theory requires in-depth studies and a higher number of patients who are followed-up for a significant period of time. However, only a few studies have been performed in this field while, to our knowledge, this is the first study exploring the levels of IL-34 in patients with AIDN and CIDP. In this regard, we think that our research adds some important knowledge to the literature.

Compared to the data on IL-34 function, the function and structure of IL-17 are much clearer. The main source of IL-17 is specialized T cells, which are called Th-17. IL-17 has 6 subtypes that are named consecutively from IL-17A to IL-17F. Among these subtypes, IL-17A and IL-17F are the two that have received the

most interest. Functionally, both of these interleukins have proinflammatory roles, albeit different with regard to inflammation type and region<sup>26</sup>. In a study by Liang et al.<sup>17</sup>, Th-17 and IL-17 were reportedly found to be involved in AIDN pathogenesis, findings which were partly confirmed by Han et al.<sup>16</sup>, who provided some evidence about the possible contribution of IL-17 on GBS pathogenesis. In another study conducted by Shujuan Li et al.<sup>27</sup>, GBS patients were found to demonstrate a significant elevation of plasma IL-17 and Th-17 concentrations; furthermore, intravenous immunoglobulin (IVIG) therapy was found to downregulate Th17 and IL-17 levels. As expected, due to their similar pathogenesis, many other studies also reported a possible relationship between IL-17 and CIDP<sup>12</sup>. For instance, Horste et al.<sup>13</sup> detected that high numbers of IL-17-producing cells were related to younger age and shorter disease duration in CIDP. Our results were consistent with the literature on this topic; however, due to the fact that CSF is a direct indicator of the physiological status of the nervous system, we evaluated IL-17A and IL-17F levels in the CSF of our patients. Considering that none of the aforementioned studies evaluated CSF IL-17 levels in both patients with AIDN and CIDP, we believe our results contribute to the literature by showing the differences (or similarities) between the diseases in terms of IL-17 levels in CSF.

There are some certain limitations in this research, i.e., the limited number of subjects; however, gathering a significant number of patients without any confounding factors, conditions or diseases can be very difficult, especially in a single center. Secondly, we could not evaluate the disease onset CSF findings of CIDP patients due to ethical concerns and also the study design. Finally, due to financial and technical limitations, we were unable to determine the levels of other cytokines, such as TNF  $\alpha$ , which may have provided further data with regard to the inflammatory characteristics of patients.

## CONCLUSION

To conclude, although we had a limited number of patients and limited resources, we believe we have addressed an important knowledge gap regarding the differences (or similarities) between patients with AIDN and CIDP in terms of IL-17A, IL-17F, and IL-34 concentrations in the CSF. Our results suggest a possible role for IL-34 in AIDN pathogenesis; however, much research is required to draw a conclusion on

this matter. However, more detailed prospective studies with a higher number of patients are required to clarify the association between IL-17, IL-34 levels, and the CIDP and AIDN.

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### Disclosure statement

No potential conflict of interest was reported by the authors.

### Ethical approval

All procedures performed in the study involving human participants were in accordance with the ethical standards of the institutional and national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. This article does not contain any studies with animals performed by any of the authors.

### Author's Contributions

The contribution of the authors is equal in all matters.

## RESUMO

**OBJETIVO:** O papel das interleucinas, como IL-17 e IL-34, na patogênese da doença auto-imune foi estabelecido na literatura. No presente estudo, objetivamos identificar as concentrações de IL-17 (IL-17A, IL-17F) e IL-34 no líquido cefalorraquidiano (LCR) de pacientes com polineuropatia desmielinizante inflamatória crônica (CIDP) e neuropatia desmielinizante inflamatória aguda (AIDN).

**MÉTODOS:** incluímos neste estudo 8 pacientes com CIDP (nenhum deles recebendo terapia imunomoduladora ou imunossupressora), 7 pacientes com síndrome de Guillain-Barre (GBS, AIDN) e 7 indivíduos controle. Os diagnósticos CIDP e AIDN foram feitos por avaliação clínica e investigações eletrofisiológicas de acordo com critérios internacionais. As amostras de LCR foram obtidas adequadamente e os níveis de IL-17A, IL-17F e IL-34 foram medidos através de kits ELISA.

**RESULTADOS:** As concentrações de IL-17A, IL-17F e IL-34 foram maiores naqueles com CIDP e AIDN em comparação aos controles ( $p = 0,005$ ,  $p = 0,01$  e  $p = 0,001$ , respectivamente). Enquanto os níveis de IL-34 foram significativamente mais altos nos pacientes com AIDN do que nos pacientes com CIDP ( $p = 0,04$ ), não houve diferenças significativas entre os grupos com AIDN e CIDP em relação aos níveis de IL-17A e IL-17F ( $p = 0,4$  e  $p = 0,2$ , respectivamente).

**CONCLUSÃO:** Nossos resultados indicam que os níveis de IL-17A, IL-17F e IL-34 podem ter um papel no CIDP e no AIDN. Além disso, a diferença nos níveis de IL-34 de pacientes com AIDN e CIDP pode indicar uma diferença importante entre a patogênese desses dois conjuntos de doenças.

**PALAVRAS-CHAVE:** Síndrome de Guillain-Barré. Citocinas. Interleukins. Interleucina-17. Interleucina-34.









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# Action of hormonal therapy in amyotrophic lateral sclerosis: a systematic review

 Kátia de Vasconcelos<sup>1</sup>  
 Acary Souza Bulle Oliveira<sup>2</sup>  
 Luiz Fernando Portugal Fuchs<sup>1</sup>  
 Ricardo Santos Simões<sup>3</sup>  
 Manuel de Jesus Simoes<sup>1</sup>  
 Manoel João Batista Castello Girão<sup>1</sup>  
 Jose Maria Soares Júnior<sup>3</sup>  
 Edmund Chada Baracat<sup>3</sup>

1. Departamento de Ginecologia, Escola Paulista de Medicina/Universidade Federal de São Paulo; São Paulo, SP, Brasil

2. Departamento de Neurologia e Neurocirurgia, Escola Paulista de Medicina/Universidade Federal de São Paulo; São Paulo, SP, Brasil

3. Departamento de Ginecologia e Obstetrícia, Hospital das Clínicas, Faculdade de Medicina da Universidade de São Paulo; São Paulo, SP, Brasil.

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## SUMMARY

*Amyotrophic Lateral Sclerosis (ALS) is a fatal disease characterized by muscle weakness, atrophy, fasciculations, and decreased reflexes due to upper and lower motor neurons death. It can be present in both sexes (55–65 years), but with predominance in males. However, in female patients, ALS presents its first symptoms when they are already postmenopausal, when then the incidence ratio of the disease is practically equal between the sexes, which leads to a probable involvement of sex hormones in the development and protection against ALS. The aim of this systematic review, which used the PRISMA consensus and NOS (New Castle–Ottawa Scale) score, was to evaluate the evidence of the action of hormone therapy in women with ALS. The Medline and Cochrane databases were accessed from March 2019 to June 2019, and only full-text articles in Spanish, English, and Portuguese were included. Only four articles matched our inclusion criteria. Postmenopausal women who used exogenous estrogen did not have the same protective factor as women still under the action of endogenous estrogen in the same age group. There was also no increase in the survival of these women.*

**KEYWORDS:** Amyotrophic Lateral Sclerosis. Gonadal Steroid Hormones. Hormone Replacement Therapy.

## INTRODUCTION

Amyotrophic Lateral Sclerosis (ALS) is a fatal neurodegenerative disease characterized by the progressive degeneration of the upper and lower motor neurons in the motor cortex and spinal cord. It may be sporadic, which corresponds to 90% of cases, or familial, mostly autosomal dominant, which corresponds to 10% of cases. The disease initially presents

itself with localized muscle weakness, progressing to stiffness and paralysis, and eventually compromising swallowing and diaphragm movements, which leads patients to die within 2 to 5 years due to respiratory failure<sup>1,3</sup>.

In both its etiology and pathogenesis, the influence of sex hormones can be noted. Some data corroborate

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CORRESPONDING AUTHOR: Manuel Simoes

Rua Botucatu, 740 – Ed. Lemos Torres – São Paulo, São Paulo – Brasil – 04021-001 – Tel: 5576 48 48 Voip 1112

E-mail: mjsimoes\_43@hotmail.com

the idea of hormonal influence, such as the later onset of the disease in women, usually around 70 years old, when they are already in menopause. While for men, the age of disease's onset is between 55 and 65 years, and cases may occur in younger individuals<sup>1,3</sup>.

Another evidence of the influence of sex hormones on the disease is the men/women ratio (2:1). Concerning the differences of individuals affected by ALS, the number of affected males is superior to that of females up until a certain stage of life, i.e., around 60 years, when most women have already entered menopause, and that's when the number of affected individuals between men and women decreases, and the ratio almost equals when we consider the aging/menopause factor for women. In cases where younger individuals were affected, we also found a high prevalence of males. Another factor is survival time, which is also longer among female patients<sup>1,2,4</sup>.

The combination of these reported factors and others lead us to believe not only in the influence of sex hormones on ALS but also in a neuroprotective action of estrogens on motor neurons<sup>1,2</sup>. Our review aims to analyze the possible protective action of hormone therapy on Amyotrophic Lateral Sclerosis.

## METHODS

This systematic review was reviewed and approved by the Research Ethics Committee (CEP) of the Federal University of São Paulo - Paulista School of Medicine (UNIFESP-EPM) under CEP number 2289090919.

The PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analysis) recommendation was used to complete the systematic review<sup>5</sup>.

### Databases

For the identification and selection of studies analyzing the ALS outcome in women using hormone therapy, the Medline and Cochrane primary electronic databases were accessed from March 2019 to June 2019. There was no restriction on the publication year. Only articles whose full text could be retrieved and that had been published in Spanish, English, or Portuguese were considered. The search strategies and mesh terms used are shown in box 1.

Medline/Pubmed: (Amyotrophic Lateral Sclerosis OR Lou-Gehrigs Disease OR Gehrigs Disease OR Amyotrophic Lateral Sclerosis, Guam Form OR Amyotrophic Lateral Sclerosis Parkinsonism Dementia Complex 1 OR Guam Disease OR Disease, Guam OR Amyotrophic

Lateral Sclerosis With Dementia OR Sclerosis, Amyotrophic Lateral OR Charcot Disease OR Motor Neuron Disease, Amyotrophic Lateral Sclerosis) AND (Gonadal Hormones OR Progesterone OR Estrogens OR Agonists, Estrogen Receptor OR Receptor Agonists, Estrogen OR Estrogen Effect OR Estrogenic Effect)

Cochrane: Amyotrophic lateral sclerosis AND Gonadal hormones

### BOX 1. SEARCH STRATEGIES AND DATABASES.

Medline/Pubmed: (Amyotrophic Lateral Sclerosis OR Lou-Gehrigs Disease OR Gehrigs Disease OR Amyotrophic Lateral Sclerosis, Guam Form OR Amyotrophic Lateral Sclerosis Parkinsonism Dementia Complex 1 OR Guam Disease OR Disease, Guam OR Amyotrophic Lateral Sclerosis With Dementia OR Sclerosis, Amyotrophic Lateral OR Charcot Disease OR Motor Neuron Disease, Amyotrophic Lateral Sclerosis) AND (Gonadal Hormones OR Progesterone OR Estrogens OR Agonists, Estrogen Receptor OR Receptor Agonists, Estrogen OR Estrogen Effect OR Estrogenic Effect)

Cochrane: Amyotrophic lateral sclerosis AND Gonadal hormones

### Article retrieval

Two researchers conducted the study selection, the evaluation of titles and abstracts obtained from the search strategies in the consulted databases, independently and blindly, obeying the established inclusion criteria and selecting the articles with potential relevance. A manual search was performed by reviewing the references (narrative or systematic) and the selected articles. Observational studies were included in the evaluation (Figure 1).

### Characteristics of selected studies and risk of bias assessment

Information on author, publication year, type of study, number of patients and gender, menarche and menopause age, use of hormone therapy, duration of hormone therapy, and mean age are shown in Table 1<sup>16-9</sup>. The assessment of the risk of bias of the studies was conducted using the Newcastle Ottawa Scale Critical Assessment Checklist (NOS), considering studies consistent when they achieved a score  $\geq 6$  and inconsistent when there was a score  $< 6$  (Table 1). The following domains of risk bias were contemplated in the assessment: patient selection (generalization and applicability), study group comparability, outcome assessment methods (cohort studies), proof of exposure (case-control), and appropriate follow-up<sup>10</sup>.



**FIGURE 1.** CHARACTERISTICS OF THE SELECTED STUDIES

| Author and publication year | Study type   | Country                         | Number of cases/controls ♀ (n)   | Mean age cases/controls, in years (±SD) | Menarche age, in years (±SD) | Menopause age, in years (±SD)               | Number of cases/control in hormone therapy (ΔT)  | NOS |
|-----------------------------|--------------|---------------------------------|----------------------------------|---|------------------------------|---|--|-----|
| Rooney et al. <sup>6</sup>  | Case-control | Netherlands<br>Ireland<br>Italy | Cases: n=653<br>Controls: n=1217 | Cases: 65.3<br>Controls: 64.7           | Cases: 13<br>Controls: 13    | Cases: 49,5 (±5,5)<br>Controls: 49,3 (±5,7) | Cases: n=93 (NA)<br>Controls: n=207 (NA)   | 8   |
| De Jong et al. <sup>7</sup> | Case-control | Netherlands                     | Cases: n=209<br>Controls: n=672  | Cases: 65.4<br>Controls: 63.4           | Cases: 13<br>Controls: 13    | Cases: 50<br>Controls: 51                   | NA   | 8   |
| Popat et al. <sup>8</sup>   | Case-control | USA                             | Cases: n=62<br>Controls: n=131   | Cases: 67,5<br>Controls: 67,6           | Cases: 13<br>Controls: 13    | Cases: 51,4<br>Controls: 52,2               | Cases: n=15 (< 8 years of HT)<br>Cases: n=21 (≥ 8 years of HT)<br>Controls: n=31 (< 8 years of HT)<br>Controls: n=32 (≥ 8 years of HT) | 9   |
| Rudnicki, <sup>9</sup>      | Case-control | USA                             | Cases: n=40<br>Controls: n=33    | Cases: 63,2±8,4<br>Controls: 60,8±10,1  | Cases: ND<br>Controls: ND    | Cases: 45,8 (±7,7)<br>Controls: 45,2 (±8,1) | Cases: n=21 (NA)<br>Controls: n=11 (NA)  | 9   |

Captions: NA: not available; SD: standard deviation; HT: hormone therapy; NOS: Newcastle–Ottawa Scale

## RESULTS

The search strategies resulted in ninety-six articles, of which only four were in line with our objectives. The main reasons that led to article exclusions were a subject not related to our objective, animal experiments, and articles written in a language other than Spanish, English, or Portuguese.

### Study characteristics

All four articles are observational studies conducted in different countries (USA, Italy, Ireland, and the Netherlands). Of these, one was a multicenter study conducted in the Netherlands, Ireland, and Italy. The number of patients analyzed in the four studies was 964 women with ALS and 2053 controls.

The two articles with the lowest number of cases and controls were the research conducted by Rudnicki<sup>9</sup>, which presented the lowest number of cases (n = 40) and controls (n = 33), and Popat et al.<sup>8</sup>, with 62 cases and 131 controls. Of the four included studies, only research conducted by De Jong et al.<sup>7</sup> did not present the number of cases and controls that used hormone therapy and for how long the therapy lasted. The age range between patients and controls in the studies analyzed was 63 to 67 years old. Postmenopausal ALS patients were defined as cases and surveyed through a medical questionnaire, their use or not of hormone therapy. The controls were postmenopausal women at risk for ALS, and through a medical questionnaire, it was surveyed whether or not they used estrogen hormone therapy. In the studies considered, it is unclear which hormone therapy

regimen was employed as well as the doses. These women had menarche within the same age group, at thirteen years of age, and menopause (naturally occurring) at between 49 and 52 years.

### Quality assessment of the selected studies

All selected studies achieved a NOS score of 8.5. The articles presented problems in the selection of controls and proof of exposure<sup>11</sup>.

### Result analysis synthesis

In each selected study, we observed the age women entered menopause (cases and controls). We also observed how many of them underwent hormone therapy and its duration, and whether or not there was a reduction in the risk of ALS among those who underwent exogenous estrogen therapy when compared to those who did not.

When observing the percentages between cases and controls, we found, in studies conducted in the United States, a higher percentage of hormone therapy use by confirmed ALS patients (52% to 58%) when compared to control patients (33% to 48%). The opposite occurred in studies conducted in European countries, where we observed greater adherence to therapy by control patients (17%) to the detriment of confirmed ALS patients (14%).

De Jong et al.<sup>7</sup>, in a study conducted in the Netherlands, did not provide the number of women who used hormone therapy and for how long they did it. In this study, they only identified the age of menarche and the occurrence of menopause, thus calculating the time of exposure to endogenous estrogen. In the same study,

the age at which patients had the first symptoms of ALS was provided, which on average was around 64 years of age, and 57% of these women developed the bulbar form of the disease, considered the most severe and rapidly evolving. Only in the study by Popat et al.<sup>8</sup>, there is mention of the period of hormone therapy usage, both by cases and controls.

Another important finding was presented by the study by Rooney et al.<sup>6</sup>, conducted in the Netherlands. There was a reduction in the risk of developing ALS with the use of hormone therapy. There are two possible hypotheses for obtaining these results, one of them being the low prevalence regarding the use of hormone therapy in the other locations of the study compared to the Netherlands, where 10.25% of the number of cases underwent estrogen therapy, whereas, in the controls, 15.9% made use of hormone therapy. Another hypothesis would be the hormone therapy formulation used by the women participating in the study. In the same study, however, when we look at data obtained from women in the USA, the opposite was observed in comparison to those located in the Netherlands. In American women, there was an increased risk of ALS when they underwent hormone therapy.

## DISCUSSION

The literature states that ALS presents itself as a disease with higher prevalence among men compared to women up until the moment these women enter menopause, when the number of cases is practically equal<sup>2,7,9</sup>. During this period, not only a drastic decrease in estrogen levels occur, but also a slight increase in the blood plasma level of androgen in postmenopausal women, produced by the ovaries or the adrenals, which may explain the tendency to equate the ALS occurrence numbers between the two sexes<sup>2,7</sup>. This leads us to believe in a possible neuroprotection by the female hormones action, especially endogenous estrogens<sup>2</sup>. Thus, our review aims to focus on the possible protective action of estrogen in the form of postmenopausal hormone therapy to reduce the risk of developing ALS<sup>6,9</sup>.

As we observe the selected studies and their differences in the use of hormone therapy between ALS cases and postmenopausal controls, there is a relatively small variation in the percentage of women who underwent or not hormone therapy. However, we found a significant difference when comparing the use of hormone therapy between studies conducted in

European countries and those conducted in the USA<sup>6,7</sup>. In European countries, there is a greater adherence to hormone therapy by control patients than among ALS cases, whereas in US studies the opposite occurred. There was greater adherence to hormone therapy by confirmed ALS cases when compared to control patients. Even with this difference between participating countries in the use of exogenous hormones, there was no reduction in the risk of developing ALS<sup>6,9</sup>.

Contrary to expectations, in some studies, ALS symptoms appeared earlier among women who used exogenous estrogen therapy. However, some studies compared the possible protective action of endogenous estrogen with the action of hormone therapy and found that women who had been on endogenous estrogen for a longer period (time between menarche and menopause) had a lower risk for ALS as well as a slightly longer survival time compared to those using exogenous hormone<sup>6,9</sup>.

According to Rooney et al.<sup>6</sup>, among the included countries, only the Netherlands presented a beneficial relationship with the use of hormone therapy, reducing the risk of amyotrophic lateral sclerosis. The reason for the difference between the three countries cited in this study is not very clear. Among the hypotheses raised, the low prevalence of hormone therapy use by other countries compared to the Netherlands is one of them. A second hypothesis would be the different formulations of hormone therapy that would be used in the Netherlands. The cases described in the Netherlands, however, are an exception among the observed studies. Studies showing a possible protective action of estrogens report endogenous estrogen exerting greater neuroprotective action than exogenous estrogen<sup>2,6,9,12</sup>.

There are some possible mechanisms of endogenous estrogen protection. One of which would be the probable preventive action of the disease onset, promoting a direct action of cell survival by preventing cell death (acting on the apoptosis cascade), increasing the release of neurotrophins, interacting with neurotransmitters, or providing antioxidant and anti-inflammatory benefits<sup>2,6,9,12</sup>.

Some in vitro studies corroborate the idea of endogenous estrogen neuroprotection, in which treatment or pretreatment with estrogen use in spinal and cortical motor neurons cell culture was observed<sup>2,6,9,12</sup>. Those studies showed that 17 $\beta$ -estradiol and 17 $\alpha$ -estradiol protected these neurons from glutamate toxicity and NO-induced cell death.

These differences lead us to deduce that there is a possible protective action conferred by estrogen, since it was observed that the protection of these women would be linked to endogenous estrogen, and at the moment these women enter postmenopause, we observed increased risk of ALS<sup>2,6,9,12</sup>.

Regarding the effectiveness or not of using hormone therapy as a protective method, when we look at the history of exogenous estrogen use as therapy, we could not observe a significant percentage of women who could have benefited from a reduced risk of ALS by using estrogen therapy, both in cases and controls, which may be related to the fact that estrogen would only have a preventive effect and was unsuccessful as a recovery treatment for cell damage. Once damage to the neurons occurred, the hormonal action would be ineffective in reversing the process<sup>2,6,9,12</sup>.

Doubts about the effectiveness of estrogen therapy in ALS cases increased interest in the research and development of new therapies. There is a large number of potential drugs that may improve the survival or slow down the disease progression in ALS patients, including the non-steroidal selective estrogen receptor modulators (SERMs) such as tamoxifen and raloxifene associated with riluzole or edaravone<sup>13-15</sup>.

Raloxifene has estrogenic properties, but it still holds promise for future use as therapy. The mechanism of how it acts towards ALS has not been fully understood; however, Tamoxifen, which has been used as adjunctive therapy for breast cancer, may slow the progression of muscle strength loss in ALS patients associated with the regular use of riluzole or edaravone<sup>15,16</sup>.

Although this systematic review presents strong

points like the large number of women evaluated, as well as the use of a consensual scale for critical evaluation of the evidence, we found an important bias in the non-inclusion of the population homogeneity regarding the information related to the use of hormone therapy (type and dosage) and how long it was used in some studies<sup>2,6,9,12</sup>.

## CONCLUSION

We can conclude from observing the data provided by the selected studies that exogenous estrogen therapy did not have the desired beneficial effect when compared to the natural preventive protection of endogenous estrogen. In addition to not reducing the risk of ALS, the exogenous hormone has not been shown to increase the time of survival of these women.

## Disclosure

On behalf of all authors, the corresponding author states that there is no conflict of interest.

## Author's Contribution

Literature review: KV; manuscript writing: KV and MJBCG; data collection: KV, MJS and ASBO; data analysis: KV, LFPPF, RSS, MJS and ASBO; manuscript reviewer: LFPPF, MJS, ASBO and MJBCG.

## Glossary

ALS: Amyotrophic Lateral Sclerosis; PRISMA: Preferred Reporting Items for Systematic Reviews and Meta-Analysis; NOS: Newcastle Ottawa Scale Critical Assessment Checklist; NO: nitric oxide; SERMs: selective estrogen receptor modulators; Ral: raloxifene

**PALAVRAS-CHAVE:** Esclerose Amiotrófica Lateral. Hormônios Esteroides Gonadais. Terapia de Reposição Hormonal.

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# Non-criteria Antiphospholipid Antibodies: a narrative review

 Andreas Funke<sup>1</sup>  
 Henrique Luiz Staub<sup>2</sup>  
 Odirlei Andre Monticiele<sup>3</sup>  
 Gustavo Guimarães Moreira Balbi<sup>4</sup>  
 Adriana Danowski<sup>5</sup>  
 Mittermayer Barreto Santiago<sup>6</sup>  
 Danieli Castro Oliveira de Andrade<sup>7</sup>  
 Jozelia Rêgo<sup>8</sup>

1. Universidade Federal do Paraná (UFPR), Hospital de Clínicas, Serviço de Reumatologia, Curitiba, PR, Brasil.
2. Pontifícia Universidade Católica do Rio Grande do Sul (PUC-RS), Escola de Medicina, Serviço de Reumatologia, Porto Alegre, RS, Brasil.
3. Universidade Federal do Rio Grande do Sul (UFRGS), Departamento de Medicina Interna, Hospital de Clínicas de Porto Alegre (HCPA), Serviço de Reumatologia, Porto Alegre, RS, Brasil.
4. Universidade Federal de Juiz de Fora (UFJF), Hospital Universitário, Serviço de Reumatologia, Juiz de Fora, MG, Brasil.
5. Hospital Federal dos Servidores do Estado (HFSE), Serviço de Reumatologia, Rio de Janeiro, RJ, Brasil.
6. Universidade Federal da Bahia (Hupes) e Escola Baiana de Medicina e Saúde Pública, Salvador, BA, Brasil.
7. Universidade de São Paulo (USP), Divisão de Reumatologia, São Paulo, SP, Brasil.
8. Universidade Federal de Goiás (UFG), Faculdade de Medicina, Serviço de Reumatologia, Goiânia, GO, Brasil.

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## SUMMARY

The 2006 Revised Sapporo Classification Criteria for Definite Antiphospholipid Syndrome included as laboratory criteria the tests for antiphospholipid antibodies whose accuracy was regarded as satisfactory according to the evidence available at that time. In practice, however, the sensitivity and specificity of these "criteria" of antiphospholipid antibodies are sometimes insufficient for identifying or ruling out antiphospholipid syndrome. It has been studied whether the accuracy of the laboratory diagnosis of the syndrome could be improved by testing for non-criteria antiphospholipid antibodies. In this work, we review evidence on the clinical associations and diagnostic value of the most commonly studied non-criteria antibodies, namely: antiphosphatidylethanolamine, anti-annexin A5, anti-prothrombin, anti-phosphatidylserine/prothrombin complex, IgA anticardiolipin, and IgG anti-domain I of the  $\beta$ 2 glycoprotein antibodies.

**KEYWORDS:** Antiphospholipid syndrome. Antibodies, Antiphospholipid. Thrombosis/diagnosis.

## INTRODUCTION

According to the 2006 Sydney revision of the 1999 Sapporo criteria, the classification of antiphospholipid syndrome (APS) requires at least one of two clinical criteria: vascular thrombosis or pregnancy morbidity, along with laboratory evidence of the presence of moderate to high titers of IgG/IgM anticardiolipin

antibodies (aCL) and/or anti- $\beta$ 2-glycoprotein I antibodies (anti- $\beta$ 2-GPI) and/or the presence of lupus anticoagulant (LA), on 2 or more occasions, at least 12 weeks apart<sup>1</sup>.

Antiphospholipid antibodies (aPL) are a heterogeneous group of autoantibodies directed against

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CORRESPONDING AUTHOR: Andreas Funke

Unidade Clínica (Uniclin), Hospital de Clínicas da UFPR - Rua General Carneiro, 181, Curitiba, PR, Brasil - 80060-900

E-mail: afunke@ufpr.br



anionic phospholipids, phospholipid-binding plasma proteins, or phospholipid-protein complexes. Apart from the conventional laboratory markers of APS mentioned above, several other autoantibodies have been described, known as non-criteria aPL, which could help to diagnose patients with clinical manifestations of APS but who are negative for the traditional antibodies and may, therefore, present the so-called “seronegative APS” (SNAPS).

The purpose of this article is to review the main characteristics and clinical relevance of the major non-criteria aPL.

## METHODS

Specialists from the Antiphospholipid Syndrome Committee of the Brazilian Society of Rheumatology reviewed the evidence on the clinical associations and relevance of the most commonly studied non-criteria aPL. Pubmed database was searched for original studies and prior reviews on non-criteria aPL published in English or Portuguese. Additional references were obtained by manually searching the available publications. Non-criteria aPL with a larger number of studies and/or greater potential routine-testing feasibility and clinical utility in the diagnosis of APS were selected by the authors for further review. Publications were reviewed for evidence on their clinical value in the assessment of patients with suspected or confirmed APS or patients with possible SNAPS. The available evidence was discussed and compiled by the authors.

## NON-CRITERIA APL

### Antiphosphatidylethanolamine antibodies

Phosphatidylethanolamine (PE), a neutral PL, is frequently the main lipid component of microbial membranes and is largely found in mitochondria. Antibodies against PE (aPE) were reported as the sole aPL in patients with thrombotic manifestations or pregnancy loss. However, screening for IgG, IgM, and IgA aPE did not increase the diagnostic yield for APS<sup>2</sup> and, according to a 2010 report, aPE did not behave as an independent risk factor for further miscarriage in patients with pregnancy losses<sup>3</sup>. Nonetheless, aPE were the aPL with the highest odds ratio for thrombosis in a survey of 270 patients. Of major interest, 63% of the 40 aPE-positive patients were negative for conventional aPL<sup>4</sup>.

The value of aPE in patients with the so-called SNAPS is a very hot topic under investigation. In a 2012 study, thin-layer chromatography immunostaining revealed the presence of aPL in 58.3% of patients with SNAPS, being aPE documented in 30.5% of such cases<sup>5</sup>.

A comprehensive review of the aPE role in patients with thromboembolic events and pregnancy morbidity was published in 2012<sup>6</sup>. Thirty years after the original description of aPE antibodies in a patient with primary APS<sup>7</sup>, the role of aPE in individuals with unexplained thrombosis and pregnancy morbidity remains controversial.

### Anti-annexin A5 antibodies

The relationship of anti-annexin A5 antibodies (aAnx A5) with thrombophilic states and pregnancy morbidity is a matter of debate. Circulating aAnx A5 has been described in APS, recurrent miscarriages, SLE (irrespective of APS), and sclerodermic digital ulcers and were also associated with thrombotic episodes in patients with SLE and APS<sup>8</sup>. In a 2006 survey of APS, SLE, and lupus-like patients, the presence aAnx A5 was not associated with thrombosis or miscarriages. The -1C→T annexin A5 mutation, nevertheless, was an independent risk factor for miscarriages<sup>9</sup>. In practical terms, testing for aAnx A5 by ELISA still lacks standardization, and not rarely the IgM isotype is missing in studies.

In a more recent Egyptian study, the presence of the IgM aAnx A5 isotype was related to APS and recurrent pregnancy loss<sup>10</sup>. These data contrast with previous information showing a link between the IgG aAnx A5 isotype and pregnancy losses in patients with primary APS<sup>11</sup>. In 2016, it was suggested that adding the IgM aAnx A5 isotype to conventional aPL assays might be useful to detect APS subsets, as well as to evaluate the risk of thrombotic recurrence<sup>12</sup>. As a whole, testing both IgG and IgM aAnx A5 isotypes seems to be appropriate<sup>10-12</sup>.

Yet regarding pregnancy morbidity, a small group (23 cases) of obstetric APS with recurrent miscarriages was studied in 1999. The frequency of IgG and IgM aAnx A5 did not differ from controls<sup>13</sup>. In 2010, traditional aPL and aAnx A5 were analyzed in 54 women with spontaneous pregnancy loss. While LA was associated with early miscarriages and aCL with fetal losses, such linkages were not confirmed for aAnx A5<sup>14</sup>.

In patients with primary APS, high IgM aAnx A5, and high IgM aCL correlated with pulmonary

embolism, while the corresponding IgG isotypes were associated with cerebral arteriopathy<sup>15</sup>. More recent studies will probably clarify the role of aAnx A5 isotypes in different clinical subsets of APS.

In 112 patients with APS, high levels of aAnx A5 were documented as compared to controls<sup>16</sup>. In another interesting study, IgG aAnx A5 (not always coincident with LA or aCL) was found to be more frequent in patients with acute cerebral ischemia than controls<sup>17</sup>.

As a whole, few studies have addressed the prevalence of aAnx A5 in patients suspected of having APS. The majority of studies looked at their frequency in patients with confirmed aPL in conventional assays or proven APS. Moreover, most of these reports included a limited number of samples<sup>8-15</sup>.

### Antibodies to Prothrombin and Phosphatidylserine/Prothrombin

Antibodies against prothrombin can be detected by ELISA either by using prothrombin-coated irradiated plates (aPT) or the phosphatidylserine/prothrombin (PS/PT) complex as the antigen. Although aPT and antibodies to PS/PT (aPS/PT) can coexist in the same patient, they appear to belong to different antibody populations<sup>18</sup>.

Several authors have evaluated the relationship between aPS/PT and APS. Atsumi et al.<sup>19</sup> reported that aPS/PT, but not aPT, were associated with the clinical manifestations of APS in 265 patients with autoimmune diseases. In addition, IgG aPS/PT were strongly associated with the presence of LA. Tsutsumi et al.<sup>20</sup> evaluated IgG aPT and IgG aPS/PT in comparison with each other and with IgG and IgM aCL, IgG  $\beta$ 2GPI-dependent aCL and LA for their association with a history of thrombosis in 139 SLE patients and concluded that all these tests, except IgM aCL, are potentially useful in the evaluation of the thrombotic risk and that although aPT and aPS/PT correlate with each other, they are not completely identical.

Zigon et al.<sup>21</sup> assessed the diagnostic value of IgG and IgM aPS/PT, tested by both an *in-house* assay and a commercial assay, in 156 patients with systemic autoimmune diseases. Both assays were highly concordant and IgG aPS/PT was the strongest independent risk factor for obstetric complications. IgG and IgM aPS/PT were associated with venous but not arterial thrombosis. In addition, there was a highly significant association between the presence of IgG/IgM aPS/PT and LA activity. The authors conclude that

aPS/PT detected by either assay represents a promising serological marker for APS<sup>21</sup>. Sanfelippo et al.<sup>22</sup> reported the presence of aPS/PT in 41 of 728 serum specimens from patients suspected of having APS who were negative for aCL and anti- $\beta$ 2GPI. Of note, among 22 patients with available medical records, 11 had a history of thrombosis. The authors suggest that aPS/PT could contribute to the diagnosis of APS in patients who present negative tests for conventional aPL.

Fabris et al.<sup>23</sup> evaluated the impact of aPS/PT testing in the first 6 months after its introduction in a clinical laboratory practice. IgG and/or IgM aPS/PT were present in 49 (11.6%) of 421 patients selected for having a medical order for this test. In addition, aPS/PT were more frequent in LA-positive than in LA-negative patients (56.1% vs. 6.4%, respectively). aPS/PT were also more prevalent than aPT in a retrospective analysis of 52 LA-positive patients (55.8% vs. 15.4%, respectively). Finally, aPS/PT were detected in about 27% of the patients with LA results that were difficult to interpret. The authors recommend the introduction of aPS/PT in the APS diagnostic process and suggest that these antibodies may be useful when the correct interpretation of LA testing is precluded by the use of oral anticoagulants<sup>23</sup>. In a systematic review of the available evidence on aPT and aPS/PT and the risk of thrombosis in APS, Sciascia et al.<sup>24</sup> found that aPS/PT were more strongly associated with venous and/or arterial thrombosis than aPT (OR 5.11 [95% C.I. 4.2-6.3] vs. 1.82 [95% C.I. 1.44-2.75], respectively) and suggested that the routine measurement of aPS/PT, but not of aPT, might be useful in assessing the thrombotic risk in patients with previous thrombosis or with SLE<sup>24</sup>.

Zigon et al.<sup>25</sup> reported a positive IgG or IgM aPS/PT test in 22 (13%) of 169 patients with APS-related obstetric manifestations. Of note, aPS/PT was the only aPL detected in 11 (6.5%) of those patients and the only aPL associated with recurrent early pregnancy loss. In this study, aPS/PT were associated with early or late pregnancy loss and with prematurity independently from other aPL<sup>25</sup>. Amengual et al.<sup>26</sup> reported the results of an international multicentric study and a subsequent validation study evaluating two IgG aPS/PT ELISA kits for the diagnosis of APS. There was an acceptable agreement between the results of both kits and the presence of IgG aPS/PT was associated with arterial and venous thrombosis (initial and validation studies) and obstetric (initial study only) manifestations of APS. According to the authors, IgG aPS/PT

should be considered for inclusion as an additional laboratory criterion for the classification of APS<sup>26</sup>.

Nakamura et al.<sup>27</sup> retrospectively assessed the value of the combined use of tests for anti-DI and IgG/IgM aPS/PT for the diagnosis of APS in a group of 157 patients. Twenty-one patients were positive for both antibodies and presented an elevated antiphospholipid score (APL-S). Of the 14 patients who were positive for aPS/PT and negative for anti-DI, 11 (79%) had APS and high APL-S values. The authors conclude that the first-line use of the combination of these two tests may contribute to the identification of patients with APS and high risk of thrombosis<sup>27</sup>.

Litvinova et al.<sup>28</sup> assessed the prevalence and significance of non-conventional aPL, including IgG/IgM aPS/PT, aPE, aAnx A5, IgA anti-β2GPI and IgG anti-DI, in a prospective cohort of patients with APS, SNAPS, asymptomatic APL carriers or presenting with a first thrombotic or obstetric event. The number of positive non-conventional tests increased with the severity of APS, and aPS/PT were significantly associated with the clinical manifestations of APS and with the presence of LA. All patients who were triple-positive for the conventional APL showed persistent aPS/PT positivity. The authors conclude that aPS/PT are of potential value as strong markers of APS and propose the use of these antibodies for risk stratification in patients in whom LA cannot be detected because of ongoing treatment with direct oral anticoagulants<sup>28</sup>.

In a recent study, Zigon et al.<sup>29</sup> evaluated the added value of some non-criteria aPL for the diagnosis of APS and found an association between IgA aCL, IgA anti-β2GPI, and IgG/IgA aPS/PT and thrombosis, and between IgA aCL, IgA anti-β2GPI, and IgG/IgM/IgA aPS/PT and obstetric events. Interestingly, among these non-criteria aPL, only aPS/PT were detected in isolation, being positive in 3% of the seronegative patients with thrombosis and 2% of the seronegative patients with pregnancy morbidity<sup>29</sup>.

#### **IgA anticardiolipin and IgA anti-β2 glycoprotein I antibodies**

Patients with IgA aPL may present classical APS clinical manifestations such as thrombotic events and pregnancy morbidity as well as non-criteria manifestations such as livedo, skin ulcers, Raynaud's phenomenon, cognitive dysfunction, epilepsy, thrombocytopenia, and heart valve disease<sup>30</sup>. Notwithstanding, neither IgA aCL nor IgA anti-β2GPI antibodies were included in the revised Sapporo criteria for the

classification of APS, possibly because of insufficient evidence and/or sub-optimal assay standardization<sup>1,30</sup>.

IgA aCL antibodies are present in up to 38% of the patients with APS and in up to 16% of the patients with SLE<sup>31</sup>. IgA anti-β2GPI antibodies, in turn, can be detected in up to 56.3% of the APS patients and in up to 30.4% of SLE patients<sup>31,32</sup>. While isolated IgA aCL positivity is very infrequent<sup>1,30</sup>, isolated IgA anti-β2GPI antibodies are detected in 3.9 to 7.9% of SLE patients<sup>33</sup> and in up to 10.6% of the patients with clinical features of APS but negative testing for the criteria aPL (SNAPS)<sup>34</sup>. A significant association between isolated IgA anti-β2GPI antibodies and thrombosis, particularly in the arterial bed, has been reported in SLE patients<sup>30,33</sup>. These patients may also develop pregnancy loss or present non-criteria manifestations of APS<sup>33</sup>.

Despite the confirmed clinical relevance of IgA anti-β2GPI antibodies, particularly among SLE patients, and their potential utility as additional prognostic indicators in confirmed APS or as diagnostic markers in SNAPS, their testing may still be poorly standardized with wide variations in testing methodology and diagnostic accuracy of commercially available laboratory kits.

#### **Anti-β2 glycoprotein I Domain I antibodies**

The revised Sapporo criteria for the classification of APS include persistent moderate-to-high titers of IgG and IgM anti-β2GPI antibodies. However, because thrombosis-associated anti-β2GPI antibodies are mainly those directed against an epitope located at domain I (DI) of β2GPI, aPL testing based on the whole β2GPI molecule might be insufficiently specific for the clinical manifestations of the syndrome<sup>1,35</sup>. IgG anti-DI occurs in up to 73.2% of the patients with APS, up to 26.7% of the SLE patients without thrombosis, and up to 11.1% of asymptomatic APL carriers<sup>36,37</sup>.

According to the results of two systematic reviews of the literature, the presence of IgG anti-DI antibodies doubles the risk of thrombosis in patients with APS<sup>32,37</sup>. Anti-DI antibodies are very specific markers of APS (specificity above 97%), particularly in patients with thrombosis<sup>38</sup>.

The usefulness and cost-effectiveness of adding anti-DI testing to the classical ("criteria") aPL panel have been questioned by some authors, since some conventional anti-β2GPI assays may already offer sensitivity and specificity at least similar to that of anti-DI antibodies<sup>31,39</sup>.

Given that clinical practice testing for the laboratory criteria for APS, particularly for LA, suffers from poor standardization and reproducibility, an interesting approach has been proposed, namely, to make first-line use of the combination of anti-DI with aPS/PT antibody testing, which in one study conveyed very high positive predictive values and acceptable sensitivity for APS<sup>27</sup>. Should these findings be confirmed in subsequent studies, this would be a very interesting and cost-effective application of anti-DI antibody testing.

## DISCUSSION AND CONCLUSIONS

Laboratory testing for aPL still consists of different and poorly standardized assays, and their clinical associations are still mostly derived from studies with a retrospective design and wide variation in sample sizes. An overview of the clinical significance and limitations of non-criteria aPL tests is shown in Table 1.

While there is no robust evidence to propose the inclusion of aPE antibodies as criteria for APS, one should not neglect the importance of testing for aPE in cases of SNAPS. Whether or not patients with sole aPE and thrombosis should be classified in the long term as having “true” APS is another question to be addressed in the future.

An association of IgG and/or IgM aAnx A5 with thrombotic or obstetric APS is not substantiated by current data, and eventual incorporation of these antibodies to APS criteria is not justified at the moment. Nevertheless, occasional testing of aAnx A5 in aPL-negative patients suspected of APS, mainly obstetric or neurologic, is a field open to discussion.

aPS/PT, but not aPT, can significantly improve the recognition of patients with APS in clinical practice and serve as a laboratory marker for the risk of thrombotic events and pregnancy morbidity, including early pregnancy loss. In addition, aPS/PT, detected by solid-phase assays, can be a surrogate marker of the presence of LA in the setting of oral anticoagulant therapy which impairs the interpretation of functional coagulation assays.

Patients with SLE and patients suspected of having APS (with or without SLE) should be tested for IgA anti-β2GPI using standardized/validated assays, either as part of the initial aPL workup (“first line”) or as a follow-up test if criteria aPL are negative. Testing for IgA aCL does not seem to provide substantial diagnostic value because their isolated positivity is infrequent.

Despite their proven role in APS pathogenesis and potentially higher specificity for the thrombotic manifestations of APS, IgG anti-DI antibodies do not consistently improve diagnostic accuracy beyond

**TABLE 1.** NON-CRITERIA APL CLINICAL EVIDENCE OVERVIEW AND WORKGROUP CONCLUSIONS

| Test           | Clinical significance*   | Main Limitations  | Eligible to be added to APS classification criteria? | Eligible for SNAPS workup? |
|----------------|--|---|--|----------------------------|
| aPE            | Association with thrombosis and gestational morbidity  | Insufficient assay standardization; inconsistent clinical value of isolated aPE | No   | Controversial              |
| aAnx A5        | Association with neurologic events and gestational morbidity   | Insufficient assay standardization; inconsistent clinical value                 | No   | No                         |
| aPT            | Association with thrombosis/pregnancy morbidity  | Weaker association with thrombosis than that of aPS/PT                          | No (aPS/PT preferred)                                | No (aPS/PT preferred)      |
| aPS/PT         | Positivity, even if isolated, is associated with thrombosis and gestational morbidity; association with severe APS | Paucity of prospective studies  | Yes** (if LA detection is not feasible)              | Yes                        |
| IgA aCL        | Association with APS clinical manifestations   | Almost always accompanied by criteria aPL; insufficient assay standardization   | No   | Controversial              |
| IgA anti-β2GPI | Positivity, even if isolated, is associated with thrombosis and pregnancy loss, particularly in patients with SLE  | Prevalence/clinical associations less studied in non-SLE populations            | Yes  | Yes                        |
| Anti-DI        | Elevated specificity and positive predictive value for thrombotic APS.   | Low rate of isolated positivity (e.g. when criteria aPL are negative).          | No**   | No                         |

aPE: antiphosphatidylethanolamine antibodies; aAnx A5: antibodies to Annexin A5; aPT: antiprothrombin antibodies; aPS/PT: antibodies to the phosphatidylserine/prothrombin complex; IgA: Immunoglobulin A; aCL: anticardiolipin antibodies; anti-β2GPI: antibodies to β2-glycoprotein I; anti-DI: antibodies to Domain I of β2-glycoprotein I \*According to available evidence, e.g., mostly retrospective and/or cross-sectional studies and systematic literature reviews of those studies \*\* First-line simplified APS workup with combined aPS/PT and anti-DI awaits further study



conventional aPL testing. Moreover, IgG Anti-DI do not appear to increase the diagnostic yield in patients with suspected SNAPS because these patients are, by definition, IgG anti- $\beta$ 2GPI negative. Therefore, routine anti-DI antibody testing adds little additional value to criteria aPL testing in suspected or confirmed APS patients.

The first-line combination of anti-DI and aPS/PT testing may provide a simplified and reliable route

for diagnosis of APS; however, more study is required before this strategy can be routinely recommended.

In patients with clinical manifestations of APS but with negative classical criteria aPL antibodies (i.e. SNAPS), the most promising non-criteria tests are IgA anti- $\beta$ 2GPI and IgG/IgM aPS/PT.

### Author's Contribution

All authors have contributed equally to this work.

## RESUMO

*A classificação de Sapporo revisada para a síndrome antifosfolípica definida de 2006 incluiu como critérios laboratoriais aqueles testes para anticorpos antifosfolípidos cuja acurácia era considerada satisfatória de acordo com a evidência então disponível. Porém, na prática, a sensibilidade e especificidade desses anticorpos antifosfolípidos "critério" são por vezes insuficientes para identificar ou descartar a síndrome antifosfolípica. Tem-se estudado se a acurácia do diagnóstico laboratorial da síndrome poderia ser melhorada por meio da testagem de anticorpos antifosfolípidos não critério. Neste trabalho revisamos a evidência a respeito das associações clínicas e valor diagnóstico dos anticorpos não critério mais estudados, nomeadamente: anticorpos antifosfatidiletanolamina, antianexina A5, antiprotrombina, anticomplexo fosfatidilserina/protrombina, IgA anticardiolipina e IgG antiodomínio I da anti- $\beta$ 2 glicoproteína I.*

**PALAVRAS-CHAVE:** Síndrome antifosfolípica. Anticorpos antifosfolípidos. Trombose/diagnóstico.

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# Smell and taste alterations in COVID-19 patients: a systematic review

 Laura Faustino Gonçalves<sup>1</sup>  
 Ana Inês Gonzáles<sup>2</sup>  
 Karina Mary Paiva<sup>3</sup>  
 Fernanda Soares Aurélio Patatt<sup>4</sup>  
 Janaina Viana Stolz<sup>5</sup>  
 Patrícia Haas<sup>6</sup>

- <sup>1</sup>. Graduanda em Fonoaudiologia pela Universidade Federal de Santa Catarina – UFSC, Florianópolis, SC, Brasil  
<sup>2</sup>. Universidade do Estado de Santa Catarina, Centro de Ciências da Saúde e do Esporte CEFID-UDESC, Núcleo de Cardiologia e Medicina do Exercício, Florianópolis, SC, Brasil  
<sup>3</sup>. Professora Doutora do Curso de Fonoaudiologia pela Universidade Federal de Santa Catarina – UFSC, Florianópolis, SC, Brasil  
<sup>4</sup>. Doutora pela Universidade de Brasília (UnB), Brasília, DF, Brasil  
<sup>5</sup>. Médica Janaina Viana Stolz Formada pela Universidade Federal de Pelotas, Pelotas, RS, Brasil  
<sup>6</sup>. Professora Doutora do Curso de Fonoaudiologia pela Universidade Federal de Santa Catarina – UFSC, Florianópolis, SC, Brasil

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## SUMMARY

**OBJECTIVE:** To present scientific evidence based on a systematic review of the literature (PRISMA) to systematize information on smell and taste alterations in patients diagnosed with COVID-19.

**METHODS:** The studies were selected through combinations based on the Medical Subject Headings (MeSH). The MEDLINE (PubMed), LILACS, SciELO, and BIREME databases were used. The search encompassed articles published from January 2010 to May 2020, with no restriction of language or localization.

**RESULTS:** A total of 665 retrieved articles had the potential for inclusion. Of these, two answered the research question, which was to verify the smell and taste alterations in patients diagnosed with COVID-19.

**CONCLUSION:** The results found in this review demonstrated that there likely is an association between self-reported smell and taste dysfunctions and COVID-19 infection in such patients.

**KEYWORDS:** Anosmia; Coronavirus infections; Smell dysfunctions; Ageusia.

## INTRODUCTION

The disease caused by the new coronavirus (COVID-19) has brought about a worldwide viral pandemic, which emerged in East Asia and quickly spread to the other continents. This infection, caused by the type-2 coronavirus, is responsible for triggering severe acute respiratory syndrome (SARS-CoV-2), and symptoms such as fever, cough, fatigue, and myalgia are usually reported<sup>1</sup>.

COVID-19 was characterized by the World Health Organization (2020) as a global pandemic and health emergency on March 11, 2020, which led to a worldwide concern<sup>2</sup>. The disease has already led to the death of more than 300,000 people, while there are more than 5 million confirmed cases worldwide<sup>3</sup>.

As COVID-19 infections spread throughout Europe, an atypical new manifestation of the disease was

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 CORRESPONDING AUTHOR: Patrícia Haas  
 Campus Universitário UFSC – Florianópolis, 121 – Brasil – 88040-900  
 E-mail: haaspatricia37@gmail.com

evidenced, with a high prevalence of individuals with reported symptoms of olfactory and gustatory alterations. Initially, neither anosmia nor ageusia was considered symptoms of COVID-19<sup>4</sup>, although these alterations typically occur in viral infections in the field of otorhinolaryngology. Currently, smell and taste alteration symptoms have been constantly reported by COVID-19 patients, indicating that the oral and nasal tissues may contain virus-host cells<sup>5</sup>.

Olfactory dysfunction (OD) may appear after infections occur in the upper respiratory tract – called postviral anosmia. However, the precise underlying pathogenesis has not been fully identified when present in COVID-19 cases<sup>6</sup>. Many viruses can lead to OD and ageusia through an inflammatory reaction in the nasal mucosa and the development of rhinorrhea. When associated with COVID-19 infections, though, these alterations seem to have peculiar characteristics, as they are not related to rhinorrhea<sup>7</sup>. The description of these otorhinolaryngological symptoms in association with COVID-19 is still scarce.

Ageusia is the loss of the functions of taste, frequently mistaken for anosmia, since the tongue can only indicate texture and distinguish the tastes perceived through smell<sup>7</sup>. It is observed that ageusia can be another one of the COVID-19 symptoms. Hence, its inclusion in data collection will help provide better information about the SARS-CoV-2 infection<sup>8</sup>.

Mao<sup>9</sup>, when analyzing the neurological manifestation frequency in 214 patients infected with COVID-19, found anosmia in 5.1% and ageusia in 5.6% of them. In Europe, studies have reported a significant detection of chemosensitive disorders in COVID-19 patients, ranging from 19.4% to 88%<sup>10,11</sup>.

Given the present scenario, this systematic review aimed to verify the already available scientific evidence on smell and taste alterations of patients diagnosed with COVID-19, seeking to answer the following research question: What olfactory and gustatory alterations occur in patients diagnosed with COVID-19?

## METHODS

### Research design and search strategies

The systematic review followed the recommendations of the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA)<sup>12</sup>. The scientific articles were searched by two independent researchers in the MEDLINE (PubMed) (<https://www.ncbi.nlm.nih.gov/pubmed/>), LILACS (<http://lilacs.bvsalud.org/>), SciELO (<http://www.scielo.br/>), and BIREME (<https://bvsalud.org/>) databases, with no restrictions of language or location; the search was carried on until May 2020. As a complement, a manual search was conducted on the references of the articles already included in the research; also, grey literature was searched in Google Scholar. The research was structured and organized according to the PICOS framework – an acronym that stands for Population, Intervention, Control, Outcomes, and Study (Table 1).

org/), SciELO (<http://www.scielo.br/>), and BIREME (<https://bvsalud.org/>) databases, with no restrictions of language or location; the search was carried on until May 2020. As a complement, a manual search was conducted on the references of the articles already included in the research; also, grey literature was searched in Google Scholar. The research was structured and organized according to the PICOS framework – an acronym that stands for Population, Intervention, Control, Outcomes, and Study (Table 1).

**TABLE 1.** DESCRIPTION OF THE PICOS COMPONENTS

| Acronym | Definition  |
|---------|---|
| P       | Patients  |
| I       | Coronavirus   |
| C       | Loss of smell and taste   |
| O       | Infection   |
| S       | Descriptive study<br>Cross-sectional study<br>Observational study |

Source: Developed by the authors.

The descriptors were selected based on the Health Sciences Descriptors (DeCS) and Medical Subject Headings (MeSH). The descriptors and Boolean operators used in the searches were: (COVID) or (taste) or (smell).

## SELECTION CRITERIA

### Inclusion criteria

We selected studies whose design was descriptive, cross-sectional, cohort, clinical, randomized, and case study. The search for articles had no restriction of language or location, encompassing studies published from January 2010 to May 2020. The inclusion and exclusion criteria developed in this research are presented in Table 2.

### Exclusion criteria

Studies designed as letters to the editor, guidelines, literature reviews, systematic reviews, meta-analyses, and abstracts were excluded. Besides these, animal research and undescribed or unclear studies were also excluded. The exclusion criteria are shown in Table 2, as well.

## DATA ANALYSIS

The data for the eligibility process of the studies were extracted using a spreadsheet developed in

**TABLE 2.** SUMMARY OF THE INCLUSION AND EXCLUSION CRITERIA

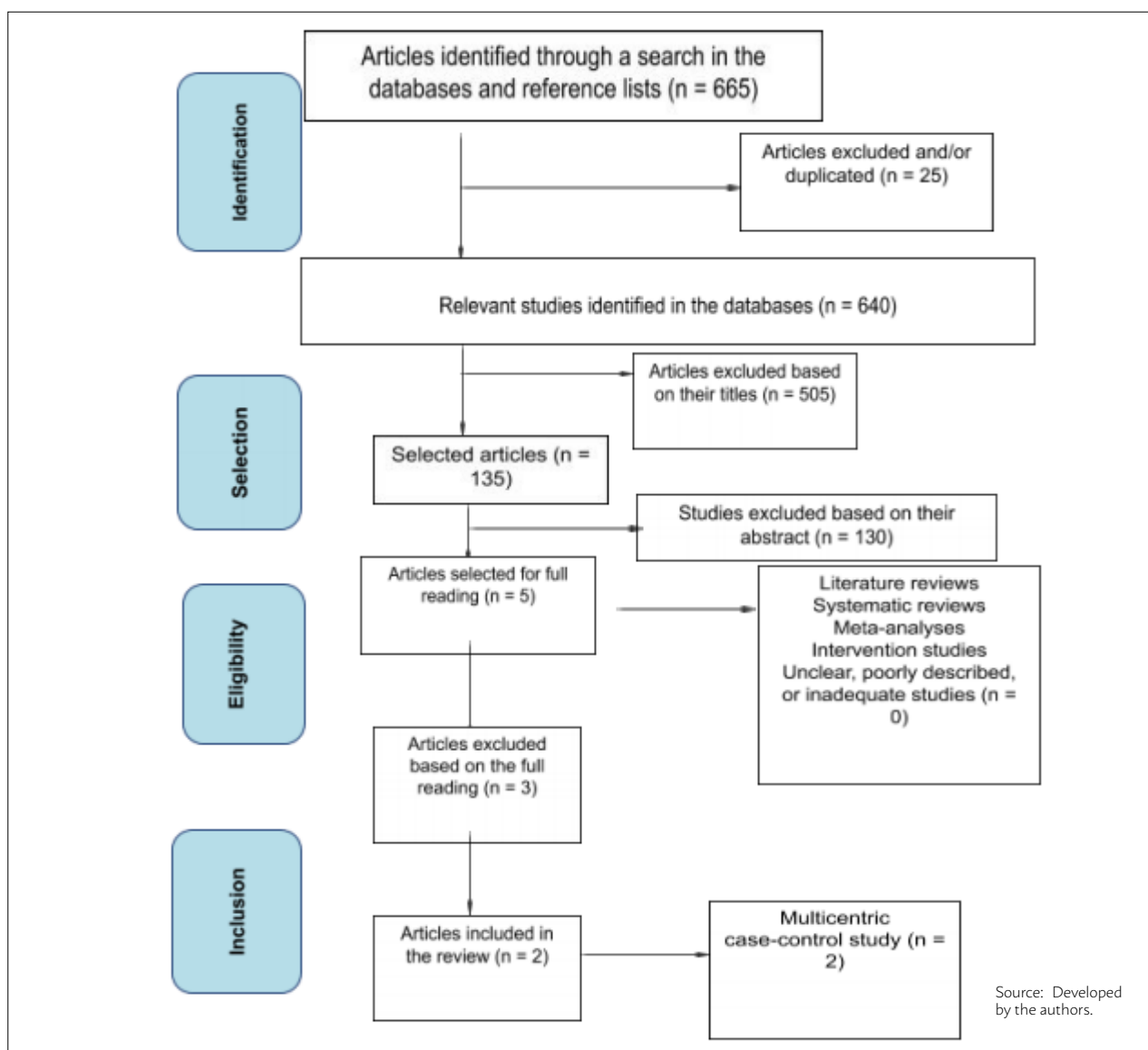
| Inclusion criteria  |  |
|---------------------|--|
| Design              | Case reports<br>Case-control studies<br>Controlled clinical trials<br>Cohort studies<br>Screening studies<br>Observational studies |
| Localization        | No restriction   |
| Language            | No restriction   |
| Exclusion criteria  |  |
| Design              | Letters to the editor<br>Guidelines<br>Literature reviews<br>Systematic reviews<br>Meta-analyses                                   |
| Studies             | Conducted with animals<br>Unclear studies<br>Inadequate or poorly described  |
| Form of publication | Only abstract  |

Source: Developed by the authors.

Excel by the researchers for systematic reviews. The extracted data were entered in the spreadsheet, initially by one of the researchers, and then conferred by another one. When necessary, a third researcher was consulted regarding the inclusion of articles. The data obtained from the eligible studies were also entered in a spreadsheet using the same software.

## RESULTS

Initially, 665 articles were selected, which were reduced to 640 after the repeated ones were excluded. Then, the titles and abstracts were analyzed, leading to the exclusion of 505 papers that did not meet the inclusion criteria; 135 articles were left. Afterward, 130 of these were excluded after having their abstracts

**FIGURE 1.** FLOWCHART OF THE SEARCH AND ANALYSIS OF THE ARTICLES

read, and the remaining five were fully read. Finally, two articles were included in the research, as they answered the research question, making them eligible according to the PRISMA criteria used to develop this research<sup>13</sup>(Figure 1).

Both studies included in this systematic review were multicentric case-control studies<sup>14,15</sup>, comprising a combined sample of 464 individuals.

The research by Beltrán-Corbellini<sup>14</sup> was developed in Spain. They administered a questionnaire regarding alterations in smell and/or taste in patients infected with COVID-19 and patients infected with the influenza virus. The questionnaires were administered during hospitalization routine clinical assessments, from March 23 to 25, 2020.

The group of patients diagnosed with COVID-19 comprised people over 18 years old who tested positive for this infection between 2019 and 2020. This study did not include specific chemosensory tests. A total of 79 patients with COVID-19 were included in the case group. The control group, in turn, comprised 46 patients with influenza virus infections (41 with A-H1; one with A-H3, four with subtype B)<sup>14</sup>.

As for the distribution of the individuals in the groups regarding gender, the number of men in the COVID-19 group was 48 (60.8%), and in the influenza group, 19 (47.5%). The mean age in the COVID-19 group was 61.6 years, and 61.1 in the influenza group. Recent-onset smell and/or taste alterations were significantly more present in the case group ( $n = 31$ ; 39.2%) than in the control group ( $n = 5$ ; 12.5%;  $p = 0.003$ )<sup>14</sup>.

As a result of the research, the authors identified that the patients with COVID-19 with recently acquired symptoms were significantly younger than the COVID-19 patients without these alterations. Of the recently acquired smell and/or taste dysfunctions reported in the case group, 25 (80.6%) had smell alterations and 28 (90.3%) taste alterations. Twenty-two patients (70.9%) had an acute onset of these dysfunctions, while in 11 (35.5%) the alterations were initial manifestations of COVID-19. Four patients (12.9%) reported concomitant nasal obstruction, and 21 patients (67.7%) were capable of distinguishing sweet and bitter, despite the dysfunctions. The smell and/or taste alterations lasted for seven days on average. In the control group, all those who had recently developed smell and/or taste alterations reported full recovery by the time they were discharged from the hospital<sup>14</sup>.

The second study<sup>15</sup> included in this research was carried out in four Italian hospitals. The olfactory and

gustatory functions were objectively assessed in two groups of patients: quarantined health professionals ( $n = 161$ ), with a positive nasopharyngeal swab for SARS-CoV-2 infection; and hospitalized patients ( $n = 184$ ) with a positive nasopharyngeal swab for SARS-CoV-2 infection. The participants' mean age was 50 years. After confirming the positive result in the first nasopharyngeal swab, variables such as gender, age, comorbidities that might be a reason for exclusion, presence and onset of symptoms of the disease were preliminarily collected.

Following the criteria proposed by Tian<sup>16</sup>, the individuals in the research<sup>15</sup> were divided into four groups according to clinical severity, namely: asymptomatic, mild (mild symptoms without radiological evidence of pneumonia), moderate (radiological evidence of pneumonia without dyspnea or respiratory insufficiency), and severe (radiological evidence of pneumonia with dyspnea and respiratory insufficiency). Regarding functional assessment in the group comprising quarantined health professionals, after testing positive for COVID-19 through the nasopharyngeal swab, the smell discrimination capacity was tested through seven groups of smells. For each one of them, the patient gave a score from 0 (no distinction) to 10 (normal distinction). The gustatory function was assessed regarding each of the primary tastes (sweet, salty, sour, and bitter) through solutions prepared by the patient, as proposed by Massarelli<sup>17</sup>.

For the group of hospitalized patients, both the olfactory threshold and the smell distinction ability were assessed. The olfactory function was assessed through the Connecticut olfactory test (CCCRC), a validated and widely used simple orthonasal olfaction test. It includes an assessment of the butanol threshold and a smell identification of 10 items, using common smells<sup>18,19</sup>. The same standardized and validated test – which investigates the capacity to perceive the four primary tastes – used with the professionals' group was also used to assess this group's gustatory function.

The study<sup>15</sup> included patients in all stages of clinical severity of the disease: 10 (2.9%) were asymptomatic; 168 (48.7%) had a mild presentation of the disease; 140 (40.6%) moderate; 27 (7.8%) severe. The chemosensitive dysfunctions during COVID-19 were self-reported by 256 patients (74.2% of the study's population); 79.3% of these patients reported combined chemosensitive dysfunctions; 8.6% olfactory dysfunctions alone; 12.1% taste alterations alone. At the time of the test,



full remission of the alteration was self-reported by 31.3% of the patients regarding smell, and by 50.4% regarding taste. The objective results obtained from the olfactory and gustatory assessments of both study groups were analyzed together.

No significant correlation was found between the gustatory and olfactory scores and the patients' gender or age. A more detailed analysis showed that there

is a significant improvement in the scores between the first and second weeks of the disease, though not between the second and the third ones. No significant correlation was found between the severity of COVID-19 and the presence or extension of chemosensitive dysfunctions. On the other hand, the duration of chemosensitive symptoms for more than seven days showed a statistically significant correlation with the

**TABLE 3.** SUMMARY OF THE RESULTS FOUND IN THE ARTICLES INCLUDED IN THE REVIEW.

| Author/Year/<br>Localization/Type<br>of study  | Purpose  | Sample  | Procedures  | Results  | Conclusion  |
|--|--|---|---|--|---|
| Beltran-Corbellini,<br>et al.<br>(2020)<br><br>Spain<br><br>Multicentric<br>case-control study | To determine whether the recent-onset smell and/or taste dysfunctions are more frequent among COVID-19 patients than in influenza patients | Case group (79): patients diagnosed with COVID-19 – 48 men and 31 women, mean age 61.6 years<br><br>Control group (40): influenza patients (41 with A-H1; 1 with A-H3; four with subtypes B) – 19 men and 21 women, mean age 61.6 years | Questionnaire on smell and/or taste alterations, administered between March 23 and 25, 2020, during hospitalization routine clinical assessments. No specific chemosensory tests were used.   | Among the patients with COVID-19 with smell and/or taste dysfunctions with recent onset, 80.6% presented alterations in smell, and 90.3%, alterations in taste; 70.9% had an acute onset of smell and/or taste dysfunctions. In 35.5%, the smell and/or taste dysfunctions were the initial COVID-19 manifestation. 12.9% reported concomitant nasal obstruction, and 67.7% were capable of distinguishing sweet and bitter, despite their smell and/or taste dysfunctions. Among the influenza patients, all those who had any recent-onset smell and/or taste dysfunctions reported full recovery. | The recent-onset smell and/or taste alterations were significantly more frequent among COVID-19 patients than among influenza patients. They usually had an acute onset and were an initial manifestation. It is suggested that the smell and/or taste alterations be assessed in the anamnesis as an indicator for COVID-19 to support their self-isolation in the present epidemic context. |
| Vaira et al.,<br>2020<br><br>Italy<br><br>Multicentric<br>case-control study                   | To investigate the smell and/or taste dysfunctions in COVID-19 patients.   | Quarantined health professionals (161). Hospitalized patients (184). The participants' mean age was 50 years.   | All the participants in the study had to present a positive nasopharyngeal swab for SARS-CoV-2 infection.<br><br>The gustatory function was assessed regarding each of the primary tastes through solutions prepared by the patient. In the group of quarantined professionals, the olfactory discrimination capacity was tested through seven smell groups; for each of them, the patient gave a score from 0 (no distinction) to 10 (normal distinction). In the group of hospitalized patients, the olfactory function was assessed through the orthonasal Connecticut olfactory test (CCCRC). | Chemosensitive disorders were self-reported by 256 patients (74.2%). Of these, 79.3% reported combined disorders; 8.6%, olfactory alone; 12.1% taste alterations alone. No significant correlations were found between the presence and severity of chemosensitive dysfunctions and the severity of the clinical condition. On the other hand, there is a significant correlation between the duration of the olfactory and gustatory symptoms and the severe progression of COVID-19  | The presence of olfactory and gustatory alterations may indicate a milder course, but it suggests that those with a more severe presentation of the disease neglect these symptoms in the condition of severe respiratory disease. This study provides more evidence of a high prevalence of self-reported smell and taste dysfunctions in association with COVID-19 infection.               |

Source: Beltran-Corbellini, et al., (2020); Vaira et al., (2020). Legend: COVID-19 = coronavirus disease 2019.

moderate and severe progression of COVID-19<sup>15</sup>. The main characteristics described in the studies included for analysis are seen in Table 3.

## DISCUSSION

Regarding the evaluation of the methodological quality of the studies eligible for this review, both obtained a score of 12 in the modified protocol by Pithon<sup>13</sup>, demonstrating their high quality.

Alterations in smell and/or taste have been recently pointed out as high-prevalence symptoms in patients diagnosed with COVID-19. The lack of objective data to quantify the extension of the problem suggests that in many countries these symptoms have not yet been included in the COVID-19 guidelines. This research aimed to verify the possible relationship between olfactory and/or gustatory alterations and the infection with the new coronavirus in patients thus diagnosed.

One of the publications comprising this study<sup>15</sup> reports that chemosensitive dysfunctions during COVID-19 were self-reported by 74.2% of the study's population. Data from both studies analyzed corroborate other research that correlates olfactory and taste alterations in COVID-19 patients<sup>18</sup>. The prevalence of chemosensitive dysfunctions in association with COVID-19 has been reported in the literature between 19.4% and 85.6%<sup>19,20,10,21</sup>.

Lechien<sup>20</sup> reported a series of 417 patients diagnosed with COVID-19 – 85.6% and 88.0% reported olfactory and gustatory dysfunction, respectively. Olfactory dysfunction appeared before other symptoms in 11.8% of the cases. Kaye<sup>22</sup> verified that of the 237 patients with COVID-19, 73% reported anosmia even before being diagnosed with the disease, and the loss of smell was the initial symptom in 26.6%.

Gustatory and olfactory dysfunctions seem to be important symptoms, standing out as clues for the diagnosis of COVID-19, particularly in the initial stage of the disease, which requires it to be acknowledged as a symptom of COVID-19 infection<sup>4</sup>. Inflammation of the nasal cavity can impair the sense of smell; it is also possible that the virus infects and damages the nasal epithelial cells<sup>23</sup>. The evidence shows that OD is highly prevalent in COVID-19, up to 80% of the patients reported subjective OD, and objective olfactory tests revealed an even higher prevalence. OD is frequently accompanied by taste dysfunction. Up to 25% of the patients with COVID-19 may present sudden onset OD as the first symptom<sup>24</sup>.

As for duration, in the study by Beltrán-Corbellini<sup>24</sup>, the symptoms lasted seven days. Another study<sup>15</sup> revealed an improvement between the first and second weeks of the disease. Other studies<sup>24,25,26,27</sup> indicate that the clinical onset of chemosensitive alterations takes place mainly in the initial stages of COVID-19 infection, usually in the first three days. However, the study by Hopkins<sup>28</sup> verified that 80% of the patients reported recovery from the loss of smell some weeks after its onset, reaching a plateau after three weeks.

Nevertheless, Vaira<sup>15</sup> reported that in patients with COVID-19, the smell and/or taste alterations are not accompanied by nasal obstruction or other rhinitis symptoms. This is probably due to the direct damage caused by the virus on the olfactory and gustatory receptors. It is not possible to determine yet whether there will be full recovery of the olfactory and gustatory functions, or how long it will take. The existence of different gustatory and olfactory recovery patterns can be explained by selective neurological impairments. The loss of taste is not a retronasal olfaction dysfunction in some patients. Further experimental studies are necessary to better understand the physiopathological mechanisms underlying the development of olfactory and gustatory dysfunctions<sup>29</sup>.

In one of the studies<sup>14</sup>, the authors used no specific chemosensory tests. In contrast, another study selected for this research<sup>14</sup> performed the nasopharyngeal swab test, olfactory discrimination capacity test, gustatory function test<sup>17</sup>, and the Connecticut olfactory test (CCCRC)<sup>18,30</sup>. The incompatibility between self-reported chemosensory alterations and psychophysical tests has already been suggested in a recent study<sup>31</sup>. Hence, the prevalence of these alterations related to COVID-19 would be overestimated in the epidemiological studies, in which the loss of smell was based on subjective reports.

## CONCLUSIONS

Although medical entities and researchers have increasingly turned their attention to the association between COVID-19 infection and possible smell and taste alterations, more detailed descriptions are necessary regarding the clinical course of this correlation.

Nonetheless, studies have already reported an association between chemosensory alterations

and COVID-19. They have also verified a significant increase in the incidence of these alterations at the onset of the disease. It is indicated that smell and/or taste dysfunction assessments be made in the anamnesis as an indicator of COVID-19. To conclude, the data

show a probable association between smell and taste alterations and COVID-19 infection in such patients.

### Author's Contribution

All authors have contributed equally to this work.

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# Comment on “Comparison of tru-cut biopsy and fine-needle aspiration cytology in an experimental alcoholic liver disease model”

 Başak Büyük<sup>1</sup>

<sup>1</sup>. Department of Histology-Embryology, Faculty of Medicine, İzmir Democracy University, İzmir, Turkey.

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Diseases of the liver are roughly divided into benign and malignant, as are many organ diseases. In addition to inflammatory conditions and congenital diseases, benign lesions include toxic conditions caused by many factors due to the obvious metabolic functions of the liver, while malignant lesions include primary and metastatic liver tumors. While biochemical and/or microbiological tests and imaging methods can guide the diagnostic approach in liver diseases, histological evaluation is often used to effectively assess the extent of damage to the liver or to obtain a definitive diagnosis<sup>1</sup>.

Samples taken for histological evaluation are at the tissue level, and cytological examinations are generally used in the diagnosis of space-occupying lesions, mostly neoplasms<sup>2</sup>. Buscarini et al.<sup>3</sup> reported that the diagnostic accuracy was significantly higher in the 2091 cytological examinations that they evaluated retrospectively in the space-occupying lesion of the liver and that the difference between histological examination and cytological examination was not statistically significant. The significance of cytological examination in diseases of the liver other than space-occupying lesions has not been clearly demonstrated, except for experimental studies<sup>4</sup>.

Biopsies performed for histological examination of the liver cause complications, including pain, bleeding, peritonitis, pneumothorax, and even death<sup>5</sup>. Studies specifying the diagnostic accuracy, positive predictive value, negative predictive value, sensitivity, and specificity of cytological examinations in many liver diseases, especially viral hepatitis, alcoholic liver disease, non-alcoholic fatty liver disease, toxic and metabolic liver diseases, are required. It is thought that cytological examinations can be used in the diagnosis and follow-up of the relevant disease if they are found to be appropriate and competent.

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CORRESPONDING AUTHOR: Başak Büyük

İzmir Demokrasi Üniversitesi Tıp Fakültesi Mehmet Ali Akman mah, 13, Sok, no.2, Konak, İzmir, Turkey

Tel: +90 505 237-2701

E-mail: drbasakbuyuk@hotmail.com

