

# Assessment of Anthropometric and Physical Health Indicators before and after Pituitary Surgery in Patients with Nonfunctioning Pituitary Adenomas, Acromegaly, and Cushing Disease

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## Abstract

**Introduction:** Individuals with pituitary adenomas may have organic consequences of their disease or therapy, and psychological changes can compromise their quality of life (QoL). This study aimed to determine the anthropometric profile and health indicators of patients with pituitary adenoma before and after pituitary surgery. **Methods:** Forty-four patients were included in this study. Out of these, 22 patients had nonfunctioning adenomas (50%), 17 acromegaly (38.6%), and 5 patients with Cushing's disease (11.4%). Anthropometric measurements included body mass index (BMI), waist circumference (WC), and waist-to-hip ratio (WHR). Health indicators included body fat percentage (BF%), basal metabolic rate (BMR), maximal oxygen uptake ( $VO_2$  max), and hand grip strength. Physical activity level (the International Physical Activity Questionnaire [IPAQ]), subjective perception of health, body image (Body Shape Questionnaire), body satisfaction (Stunkard Figure Rating Scale) were used. **Results:** The mean patient age was  $47.2 \pm 14.6$  years; of which 25 were women (56.8%). Before surgery, 75.0% were overweight or obese, 84.1% had WC with risk of metabolic complications, and 90.0% had WHR with cardiovascular risk. There was a high BF% in 56.4% of cases, low BMR in 65.1%, lower  $VO_2$  max in 16.2%, and below-average grip strength in 88.6%. Hypopituitary patients had poorer cardiorespiratory fitness. The IPAQ showed reduction in physical activity, and 79.5% of patients were dissatisfied with their body image. Patients with nonfunctioning adenomas had better perception of their health while those with Cushing's disease had more distorted body image. Postoperatively, patients with acromegaly showed improvement in WHR and physical activity level, and patients with Cushing's disease showed improvement in anthropometric variables. **Conclusions:** These findings emphasize the need for continuous monitoring of this population for anthropometric indicators associated with metabolic and cardiovascular comorbidities as well as body satisfaction.

**Keywords:** Acromegaly, ACTH-secreting, adenoma, pituitary

## INTRODUCTION

Pituitary adenomas represent 10–15% of all intracranial tumors. [1] These adenomas are associated with clinical syndromes, such as acromegaly, Cushing's disease, and amenorrhea-galactorrhea syndrome, or they may be clinically nonfunctioning. Tumors secreting growth hormone (GH) and adrenocorticotropic hormone (ACTH) are associated with increased morbidity and mortality. [2–4] Either because of the disease or its treatment, pituitary tumors may be accompanied by psychological disorders that affect the patient's quality of life (QoL). [5] The

present study aimed to determine the anthropometric profile and health indicators, including QoL, of patients with pituitary adenomas before and after pituitary surgery.

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## METHODS

This is a prospective, longitudinal study of patients diagnosed with pituitary adenoma, who underwent pituitary surgery performed by the same surgeon over a period of 34 months. The study was conducted at a neurosurgical reference hospital in the South of Brazil. It was approved by the institution's research ethics committee and was conducted in compliance with the Declaration of Helsinki. Prior to inclusion in the study, all the patients signed informed consent, and their privacy was preserved.

The study population consisted of 44 patients, aged 21 to 74 years. They were evaluated during the preoperative period and reevaluated at least 4 months after the procedure. The comorbidities presented by the patients were hypertension, dyslipidemia, diabetes mellitus, depression, and occasionally another specific diagnosis, all under treatment, without compromising the physical activity at the time of the evaluation either in the pre- or postoperative period.

The diagnosis of the tumor type was made on the basis of clinical, biochemical, and histological findings. Based on the size, pituitary adenomas were classified into microadenomas (lesions <1 cm in diameter) and macroadenomas (lesions  $\geq$ 1 cm in diameter).<sup>[6]</sup> Hypopituitarism was defined as deficiency of one or more pituitary hormones. At the time of the pre- and postoperative evaluations, all patients were receiving adequate replacement therapy for the deficient pituitary axes, except for the GH axis.

Anthropometric measurements were taken by a trained professional who followed the standard protocols. The height and weight were measured using a Welmy® scale with participants standing upright, wearing light clothing, and no shoes. The Body mass index (BMI) was calculated as current weight (kg)/height (m<sup>2</sup>), and the patients were classified as underweight (<18.5), normal weight (18.5–24.9), overweight ( $\geq$ 25.0), or obese ( $\geq$ 30.0). Obesity was classified as grade I (30–34.9), grade II (35–39.9), or grade III ( $\geq$ 40). Body circumferences were measured with the patient standing upright, using a flexible, nonstretchable tape measure accurate to 0.1 cm (Sanny®). The waist circumference (WC) was measured as the maximum abdominal girth between the lowest rib and the iliac crest, and it was categorized by the risk of metabolic complications as increased (men  $\geq$  94 cm, women  $\geq$  80 cm) or greatly increased (men  $\geq$  102 cm, women  $\geq$  88 cm). The WC was measured at the end of normal expiration at the midpoint between the lower border of the rib cage and iliac crest, and the hip circumference (HC) was measured at the level of the widest circumference over the greater trochanters. The waist-to-hip ratio (WHR) was calculated and categorized by cardiovascular risk as low, moderate, high, or very high. The anthropometric measurements were classified according to the World Health Organization (WHO).<sup>[7,8]</sup>

The health indicators were measured by tetrapolar wrist–ankle bioelectrical impedance analysis (BIA) using a Biodynamics

Body Composition Analyzer (model 310; Biodynamics Corp., Seattle, WA, USA). Body composition was categorized by the body fat percentage (BF%) as very poor, poor, below average, average, above average, good, or excellent;<sup>[9]</sup> basal metabolic rate (BMR), was categorized by sex and age as low, medium, or high; and estimated maximal oxygen uptake (VO<sub>2</sub> max), was calculated as  $(57.50 - 0.31[X1] - 0.37[X2])$ , where X1 = age in years and X2 = BF% and categorized by cardiorespiratory fitness as very poor, poor, fair, good, excellent, or superior.<sup>[10]</sup>

The dominant-hand grip strength was measured using a hand dynamometer with adjustable handle spacings (Baseline®). The patients were seated comfortably on a chair without armrests, with their feet on the ground, and instructed to squeeze the dynamometer as hard as possible with the dominant hand three consecutive times, with a 30-second interval between trials. Then, the best of the three readings was recorded and categorized by sex and age as below or above average.<sup>[11,12]</sup> The physical activity level was assessed using the International Physical Activity Questionnaire (IPAQ), and the patients were classified as active, minimally active, or inactive.<sup>[13]</sup> Subjective perception of health was assessed by the question, “How is your health?”, with five response options: very poor, poor, neither poor nor good, good, and very good.<sup>[14]</sup> The self-report Body Shape Questionnaire (BSQ) was used to assess body image perception, which was categorized in relation to distortion of body image as none, mild, moderate, or severe.<sup>[15]</sup> The level of body satisfaction was assessed using the Portuguese version of the Stunkard Figure Rating Scale (FRS),<sup>[16]</sup> and patients were classified as satisfied or dissatisfied.

Quantitative data were expressed as mean (SD) and categorical data as counts and percentages. Ordinal scores were summarized as mean scores and counts and percentages. Student's *t* test was used to compare the means of quantitative variables. Binary categorical data were compared using Fisher's exact test. Scores were compared between two groups using the Mann-Whitney test and between three groups using the Kruskal-Wallis test. Paired equivalent tests were used for before-and-after comparisons. The level of significance was set at  $P < 0.05$ . Data were processed and analyzed using SPSS, version 22.0.

## RESULTS

The sample consisted of 19 men (43.2%) and 25 women (56.8%), with a mean (SD) age of 47.2 (14.6) years. Three patients, two males and one female, refused to participate in the postoperative assessment. Twenty-two tumors were nonfunctioning adenomas (50%), 17 were GH-secreting adenomas associated with acromegaly (38.6%), and 5 were adrenocorticotrophic hormone (ACTH)-secreting adenomas associated with Cushing's disease (11.4%). In terms of the tumor size, 41 were macroadenomas and 3 were microadenomas (one case of acromegaly and two cases of Cushing's disease). Hypopituitarism was diagnosed in 27 patients: hypothyroidism, hypocortisolism, hypogonadism, and GH deficiency were

present in 18, 13, 20, and 12 patients, respectively. The number of axes affected was one in eight cases, two in seven, three in seven, and four in five cases.

**Preoperative evaluation**

The characteristics of patients evaluated before pituitary surgery are shown in Table 1.

According to BMI, one patient was underweight (2.3%), 10 had normal weight (22.7%), 18 were overweight (40.9%), and 15 were obese (43.1%) (11 with class I obesity, 2 with class II obesity, and 2 with class III obesity). Regarding WC, 37 patients were at the risk of metabolic complications (84.1%)—12 were at increased risk (27.3%) and 25 at greatly increased risk (56.8%). Based on the WHR, 90% of patients had cardiovascular risk profiles—7 with moderate risk (15.9%), 8 with high risk (18.2%), and 26 with very high risk (56%).

Seventeen patients (43.6%) had adequate BF% values. It was increased in 22 patients (56.4%)—13 had very poor body composition, 6 had poor body composition, and 3 had below-average values. In four men, BF% could not be categorized because the classification did not cover the patient’s age. In another case, the patient had a pacemaker that precluded the performance of BIA, and we could not measure BF%, BMR, nor VO<sub>2</sub> max. These exclusions are

also valid for postoperative assessment. BMR was low in 28 patients (65.1%) and high in 15 (34.9%). Based on the VO<sub>2</sub> max, 7 patients had poor cardiorespiratory fitness (16.2%) and 13 had a fair fitness level (30.2%). The IPAQ showed a reduction in physical activity. Regarding grip strength, 39 patients (88.6%) had below-average strength.

In analysis stratified by sex, WHR (*P* = 0.01), BF% (*P* = 0.002), BMR (*P* = 0.001), and handgrip strength (*P* = 0.005) were significantly different between men and women. Regarding qualitative health indicators, only FRS scores were different between men and women (*P* = 0.003), with women being more dissatisfied with their body image than men.

Among the variables evaluated, only VO<sub>2</sub> max showed a significant difference between patients with (30.2 ± 4.9) and without (33.4 ± 5.2) hypopituitarism (*P* = 0.05), showing that patients with hypopituitarism had a poorer fitness level.

After stratifying patients according to the functional state of the tumors, difference was observed only in the qualitative health indicators, patients with nonfunctioning adenomas presenting a better perception of their health than those with acromegaly (*P* = 0.04) or Cushing’s disease (*P* = 0.05). According to BSQ scores, Cushing’s disease patients had a more distorted body image than those with nonfunctioning adenomas (*P* = 0.05) and those with acromegaly (*P* = 0.02).

**Table 1: Preoperative and postoperative characteristics stratified by sex**

Indicator	Preoperative		Postoperative	
	Men (n=19)	Women (n=25)	Men (n=17)	Women (n=24)
Age (years)*	53.4±14.9	42.5±13.2	55.6±14.1	42.2±13.4
Tumor type				
Nonfunctioning adenoma	13 (59.1%)	9 (40.9%)	13 (59.1%)	9 (40.9%)
Acromegaly	6 (35.2%)	11 (64.7%)	4 (28.6%)	10 (71.4%)
Cushing disease	-	5 (100%)	-	5 (100%)
Hypopituitarism				
Yes	18 (66.6%)	9 (33.3%)	13 (54.2%)	11 (45.8%)
No	2 (11.7%)	15 (88.2%)	2 (13.3%)	13 (86.7%)
IPAQ				
Active	10 (50%)	10 (50%)	12 (60.0%)	8 (40.0%)
Minimally active	5 (38.4%)	8 (61.5%)	1 (25.0%)	4 (75.0%)
Inactive	4 (36.3%)	7 (63.6%)	4 (23.5%)	13 (76.5%)
Subjective perception of health				
Poor	3 (42%)	4 (58%)	1 (50.0%)	1 (50.0%)
Neither poor nor good	5 (31.2%)	11 (68.7%)	7 (58.3%)	5 (41.7%)
Good	11 (57.8%)	8 (42.2%)	7 (30.4%)	16 (69.6%)
Very good	-	2 (100%)	2 (50.0%)	2 (50.0%)
BSQ				
No body image distortion	18 (50%)	18 (50%)	16 (44.4%)	20 (55.6%)
Mild distortion	1 (20%)	4 (80%)	-	2 (100.0%)
Moderate distortion	-	3 (100%)	1 (25.0%)	2 (75.0%)
FRS				
Satisfied with their body image	8 (88.8%)	1 (11.2%)	4 (80.0%)	1 (20.0%)
Dissatisfied because of underweight	1 (50%)	1 (50%)	2 (66.6%)	1 (33.4%)
Dissatisfied because of overweight	10 (30.3%)	23 (69.6%)	11 (33.4%)	22 (66.6%)

\*Mean±SD. IPAQ: International Physical Activity Questionnaire, BSQ: Body Shape Questionnaire, FRS: Stunkard Figure Rating Scale

## Postoperative evaluation

The characteristics of patients evaluated after pituitary surgery are shown in Table 1. No postoperative complications, such as rhinorrhea or other conditions that compromise physical activity, were observed.

According to BMI, 8 patients had normal weight (19.5%), 16 were overweight (39%), and 17 were obese (41.5%) (13 with class I obesity, 3 with class II obesity, and one with class III obesity). Regarding WC, 37 patients were at risk of metabolic complications—8 were at increased risk (19.5%) and 29 were at greatly increased risk (70.7%). Based on the WHR, 95% of patients had cardiovascular risk profiles: 4 with low risk (9.7%), 7 with moderate risk (17.1%), 9 with high risk (21.9%), and 19 with very high risk (46.3%).

BF% was increased in 24 patients (64.9%): 15 had very poor body composition, 5 had poor body composition, and 4 had below-average values. BMR was low in 17 patients (42.5%). Based on the  $VO_2$  max, 7 patients had fair cardiorespiratory fitness (17.5%). Regarding grip strength, 31 patients (75.6%) had below-average strength.

In analysis stratified by sex, WHR ( $P = 0.012$ ), BF% ( $P = 0.005$ ), BMR ( $P = 0.001$ ), and handgrip strength ( $P = 0.001$ ) were significantly different between men and women. Regarding qualitative health indicators, IPAQ scores ( $P = 0.02$ ) and FRS scores ( $P = 0.03$ ) were different between men and women, with perceived health and body image being worse in women. When patients with and without hypopituitarism were compared, only  $VO_2$  max showed a significant difference,  $29.8 \pm 4.6$  in those with hypopituitarism and  $33.3 \pm 5.1$  in those without ( $P = 0.03$ ). No differences were observed between patients with functioning versus nonfunctioning tumors in any of the parameters measured postoperatively.

## Preoperative versus postoperative measurements

The comparison of preoperative and postoperative data is shown in Table 2. Overall, there was a significant difference between the two moments for WHR ( $P = 0.009$ ), which was lower after surgery, but it remained within the range of high cardiovascular risk. In the same way, the subjective perception of health was better after surgery ( $P = 0.04$ ), but it remained within neither poor nor good category.

Patients with nonfunctioning adenomas showed less physical activity postoperatively ( $1.8 \pm 0.9$  vs  $1.6 \pm 0.8$ ,  $P = 0.03$ ) still within the range of active individuals according to the IPAQ scores. Patients with acromegaly had a lower WHR after surgery ( $0.9 \pm 0.7$  vs  $0.95 \pm 0.5$ ,  $P = 0.02$ ), but remaining within the range of very high cardiovascular risk, and lower IPAQ scores ( $2.1 \pm 1$  vs  $1.8 \pm 0.8$ ,  $P = 0.05$ ), with reduced physical activity after surgery. Patients with the Cushing's disease had a lower mean BMI after surgery ( $29.9 \pm 6.1$  vs  $32 \pm 7.7$ ,  $P = 0.006$ ), decreasing from the obese to the overweight range; lower WC ( $98.4 \pm 14.4$  vs  $102.6 \pm 15.2$ ,  $P = 0.004$ ), still in the range of greatly increased risk of metabolic complications; and

**Table 2: Comparison of preoperative and postoperative measurements**

Indicator	Preoperative (n=44)	Postoperative (n=41)	P
BMI (kg/m <sup>2</sup> )	28.5±5.0	28.9±4.7	0.72
AC (cm)	96.6±9.1	98.7±11.9	0.21
WHR	0.95±0.1	0.93±0.1	0.009*
BF%	30.3±7.5	31.2±7.0	0.37
BMR (kcal/day)	1685.0±272.7	1657.6±282.8	0.52
$VO_2$ max (mL/kg/min)	31.5±5.3	31.5±5.1	0.29
Hand grip strength (kg)	24.4±9.7	26.7±10.5	0.14
IPAQ	1.8±0.8	1.9±0.9	0.32
Subjective perception of health	3.4±0.8	3.7±0.7	0.04*
BSQ	1.2±0.6	1.2±0.5	0.45
FRS	2.5±0.8	2.7±0.7	0.32

Mean±SD; \* $P < 0.05$ . BMI: Body mass index, WC: Waist circumference, WHR: Waist-to-hip ratio, BF%: Body fat percentage, BMR: Basal metabolic rate,  $VO_2$  max: Maximal oxygen uptake, IPAQ: International Physical Activity Questionnaire, BSQ: Body Shape Questionnaire, FRS: Stunkard Figure Rating Scale

lower WHR ( $0.87 \pm 0.8$  vs  $0.89 \pm 0.9$ ,  $P = 0.001$ ), remaining within the range of high cardiovascular risk.

## Comparison between cured and uncured patients

Patients with functioning adenomas normalized the hormonal hypersecretion in 42.1% of cases (five cases of Cushing's disease and three of acromegaly). At the postoperative imaging follow-up, the patients with nonfunctioning adenomas showed a total tumor reduction in 31.8% of cases. No difference in any of the parameters measured postoperatively was observed between patients with hormonal hypersecretion resolution and/or complete tumor resection. Among patients with hypopituitarism, in three cases, the recovery of the pituitary function was observed for at least one of the deficient axes (gonadal axis in two cases and cortisol axis in one case). In three other cases, reassessment revealed new pituitary hormone deficiency.

## DISCUSSION

There are few reports in the literature involving the variables here analyzed in the patients with pituitary adenoma. The evaluation of quantitative variables is usually restricted to BMI. Regarding the qualitative variables, studies on the QoL show that it is compromised in secretory pituitary tumors,<sup>[17]</sup> especially in acromegaly,<sup>[18]</sup> Cushing's disease,<sup>[19]</sup> and nonfunctioning tumors,<sup>[20]</sup> and aggravated by hypopituitarism.<sup>[21]</sup>

The estimated prevalence of BMI  $\geq 25$  in the healthy Brazilian population is 53.8% while that of obesity is 18.9%.<sup>[22]</sup> In the preoperative anthropometric evaluation, we observed a high prevalence of obesity (43.1%) and overweight (40.9%), regardless of the type of adenoma or presence of hypopituitarism. These numbers are higher than in the general population and similar to that of Deepak *et al.*<sup>[23]</sup>



who evaluated 152 adults with hypothalamic-pituitary disease, 89 with nonfunctioning adenomas and adenomas associated with acromegaly and Cushing's disease, and reported obesity in 50% of the sample and overweight in 30.8%. Kim *et al.*<sup>[24]</sup> evaluated 40 men with hypopituitarism, and 70% of them had pituitary adenoma. They reported an average BMI of 25.3, close to our finding of 27.5. Schmid *et al.*<sup>[25]</sup> investigated 399 adenomas and found that 22.2% of patients with Cushing's disease, 21.4% of patients with acromegaly, and 17% of patients with clinically nonfunctioning adenomas were obese. Harbeck *et al.*<sup>[26]</sup> observed a higher prevalence of BMI  $\geq 25$  in prolactinomas (70%). In the present study, a higher percentage of obesity was found in patients with acromegaly (46.7%). It has already been observed that more often patients with macroadenomas are significantly overweight and obese than individuals with microadenomas.<sup>[26]</sup> Perhaps this finding may explain the higher frequency of obesity in acromegalics in this series, 94.1% of them with macroadenomas, in relation to individuals with Cushing's disease, 60% with macroadenomas.

The WC shows good correlation with the abdominal visceral adipose tissue and is a reliable indicator in the assessment of cardiovascular risk.<sup>[27]</sup> Deepak *et al.*<sup>[23]</sup> related central adiposity in 86% of the evaluated cases, with mean WC of  $103.4 \pm 13.9$ , and WHR in women of  $0.87 \pm 0.07$  and in men of  $0.97 \pm 0.06$ . Our WC results showed that 84.1% of patients were at risk for metabolic complications and WHR results showed that 90% were at cardiovascular risk. The presence of hypopituitarism did not interfere with the results of WC and WHR in the current series, although the small number of hypopituitary patients (13) may be a limiting factor of this analysis. Kim *et al.*<sup>[24]</sup> observed higher WC ( $87.5 \pm 0.77$ ) e WHR ( $0.91 \pm 0.01$ ) values in patients with hypopituitarism than in healthy controls.

The increase in body fat observed here ( $30.3 \pm 7.5$ ), also reported by Deepak *et al.*,<sup>[23]</sup> of  $41.9 \pm 6.9$  in women and  $29.3 \pm 6.8$  in men, reinforces the metabolic risk scenario already represented by hormonal hypersecretion per se.

In individuals without pituitary disease, comparing BMI, WC, WHR, e BF%, neither of them was consistently the strongest predictor of components of the metabolic syndrome.<sup>[28]</sup>

The assessment of aerobic capacity in patients with GHD showed significantly lower  $VO_2 \text{ max}$ <sup>[28]</sup> and no significant change 6 months after the GH replacement.<sup>[29]</sup> We observed lower cardiorespiratory fitness in patients with hypopituitarism, which is in accord with the literature. Olcyk *et al.*<sup>[30]</sup> assessed exercise capacity using the 6-minute walk test and the Borg Rating of Perceived Exertion (RPE) scale and observed decreased cardiorespiratory fitness in patients with hypopituitarism. Conceição *et al.*,<sup>[31]</sup> evaluating 26 patients with hypopituitarism, also observed decreased functional capacity.

Reduction in grip strength as observed here was reported by Füchtbauer *et al.*<sup>[32]</sup> in patients with acromegaly. Hatipoglu

*et al.*<sup>[33]</sup> reported that in acromegalics the reduction in muscle strength did not change significantly with participation in a prescheduled program of exercise.

In the present study, 45.4% of patients were considered physically active before surgery, a number similar to that of the healthy Brazilian population (35.3%).<sup>[22]</sup> Dantas *et al.*<sup>[34]</sup> found decreased levels of physical activity in patients with acromegaly, which was not observed in the present study.

Subjective perception of health was mostly good or very good. Studies involving Brazilian adults,<sup>[35]</sup> although in selected and limited samples, gave results similar to those found here. Patients with secretory adenomas, especially with Cushing's disease, showed worse health perception than those with nonfunctioning adenomas. Regarding body image perception (BSQ scores), patients with Cushing's disease experienced the greatest body image distortion, as observed by Alcalar *et al.*<sup>[36]</sup> Conaglen *et al.*<sup>[37]</sup> found no difference in body image disturbance between patients with nonfunctioning adenomas and acromegaly. Pantanetti *et al.*,<sup>[38]</sup> however, observed impaired self-esteem and body image distortion in patients with acromegaly.

The only significant differences in the postoperative period compared to the previous ones were in the WHR, especially in acromegalic patients and in patients with Cushing's disease, and health perception, both indices showing improvement. The improvement was not sufficient to reposition them in the classification bands, which could occur if the sample were enlarged. There were no statistically significant differences in patients with resolution of hormonal hypersecretion and absence of tumor residue in any of the parameters measured postoperatively (anthropometric indicators, health indicators measured by BIA, and qualitative health indicators).

In conclusion, this sample of patients with pituitary adenomas showed a high prevalence of overweight and obesity, as well as increased body fat, with consequent increased risk of metabolic complications and an underlying increase in cardiovascular risk associated with increased WHR. Most individuals were considered relatively active, perceived having good health and expressed no body image distortion, despite being dissatisfied with their body image. When evaluated according to the type of adenoma, patients with nonfunctioning adenomas had a better perception of their health and patients with Cushing's disease had a more distorted body image. The postoperative evaluation revealed no substantial changes of clinical significance, except for those related to weight in patients with Cushing's disease, subgroup in which all patients presented remission of the disease. These findings reinforce the need for continuous monitoring of health indicators and body image. The variables analyzed in this study remain underexplored in the endocrine literature and require further investigation.

#### Declaration of patient consent

The authors certify that they have obtained all appropriate patient consent forms. In the form, the patient(s) has/have

given his/her/their consent for his/her/their images and other clinical information to be reported in the journal. The patients understand that their names and initials will not be published and due efforts will be made to conceal their identity, but anonymity cannot be guaranteed.

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### Conflicts of interest

Cyro Garcia Soares Leães, Matheus Vanzin Fernandes, Luiza Alves, Bruna Araújo, Carolina Garcia Soares Leães Rech, Nelson Pires Ferreira, Luis Henrique Telles da Rosa, Júlia Fernanda Semmelmann Pereira-Lima, Miriam da Costa Oliveira have no conflicts of interest to declare.

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