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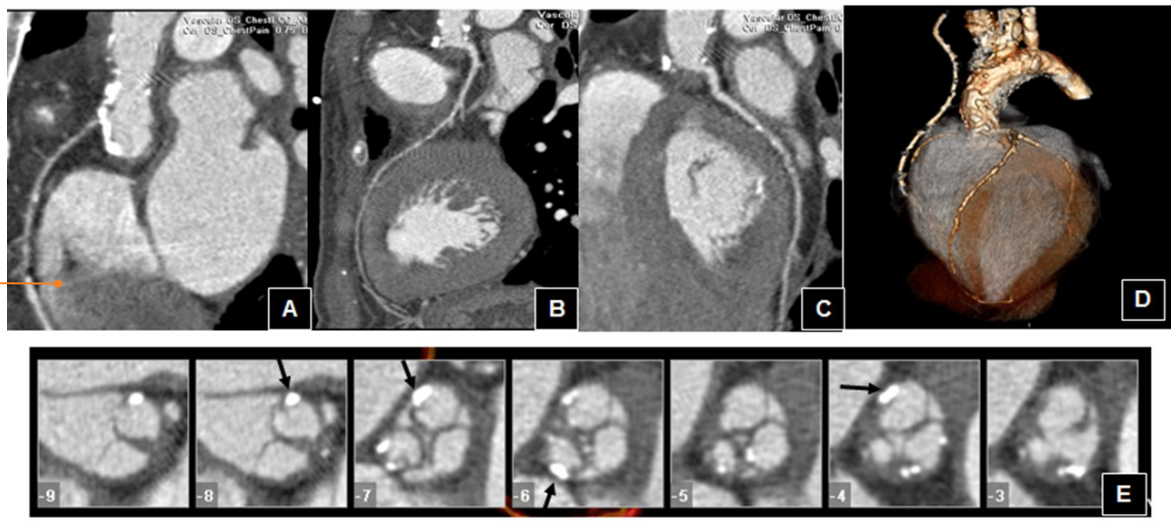
## SECTIONS



## ARTICLES





RAMB REVISTA DA ASSOCIAÇÃO MÉDICA BRASILEIRA
JOURNAL OF THE BRAZILIAN MEDICAL ASSOCIATION

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## RAMB - REVISTA DA ASSOCIAÇÃO MÉDICA BRASILEIRA (JOURNAL OF THE BRAZILIAN MEDICAL ASSOCIATION)

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EDITORIAL

# The early mobilization for children in Pediatric Intensive Care

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The definition of early mobilization (EM) in pediatrics is recent[1]. "EM was defined as the implementation of therapeutic interventions aimed at patients within 72h of their Pediatric Intensive Care (PICU) stay, including those patients on positive pressure and mechanical ventilation". However, to affirm that EM is a set of interventions to promote walking may not be adequate for pediatrics, since the age range of admission to PICU usually varies from 1 month to 17 years of age, and ambulation is not the ultimate goal for infants, for example. Thus, it is suggested to use a "set of interventions aimed at mobility"[1].

Differently from adult patients, children are more complex from the point of view of mobilization and mobility interventions, since their chronological age, cognitive maturity, and level of sedation must be respected. These characteristics associated with the variability of pathophysiologies, which can affect seriously ill children, denote a heterogeneous population, which requires specific protocols of evidence-based EM[2].

Some barriers have been reported to the clinical condition of the patient, such as clinical instability; difficulty in the clinical diagnosis and the severity of the disease; risk of displacement of devices (eg catheters and intra-tracheal cannula); excessive and/or inadequate analgesia dosage; physical constraints; obesity; inadequate nutritional status; lack of motivation in some children to participate in EM activities; among others[3,4]. However, current studies[3-6] show that multi-professional performance and family involvement contribute to the positive outcomes of EM as well as to minimize the mobilization barriers of sick children.

It is recommended that all PICU patients be evaluated by a physiotherapist regarding the possibility of participating in an EM protocol at admission at the Unit[7], as well as its beginning within three days of the patient's stay at the PICU, with levels/degrees of complexity according to their clinical condition and functional capacity[1,2].

Studies[1,8,9] have demonstrated that EM provides an increase in physical function, reduction of PICU length of stay, reduction in the mechanical ventilation time and delirium frequency, improvement of the sleep-wake cycle, reduction of hospitalization costs,

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increased family satisfaction, increased satisfaction of the multi-professional team.

It is suggested that some evaluation and monitoring measures be put in place, such as scales that contemplate muscular strength and mobility (body function), like the Medical Research Council (MRC) scale score and handheld dynamometers; and motor/cognitive ones, like the Functional Status Score for the ICU (FSS-ICU)[10]; the evaluation of the level of sedation, such as the Ramsay scale or COMFORT, aiming at optimizing sedation and avoiding delirium (which can be evaluated by the all-critically ill children for delirium using the Preschool Confusion Assessment Method (psCAM, 6 months to 5 years) (CAPD; all ages) or the Pediatric Confusion Assessment Method (pCAM, 5 years and older), to effectively identify both hypoactive and hyperactive delirium[7].

Monitoring of other markers, such as serum lactate and creatine phosphokinase, may be necessary in more severe cases in which there is no positive evolution (gain of functional independence) or in those with functional worsening after the start of the EM program. Nutritional and electrolyte evaluation (especially of calcium, sodium, magnesium, phosphorus, and vitamin D, which present correction with loss of mass and muscular function) should be considered in these cases. These measures are not yet fully defined in the literature, and clinical studies are needed.

In conclusion, several aspects, mainly related to the markers of increase in motor performance, mobility, and functionality, especially for infants and preschoolers, require studies to define secure protocols of EM in PICU.

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# Neonatal sepsis with neutropenia: granulocyte-colony stimulating factor (G-CSF)

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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.*

Sepsis is a serious complication in newborns and an important cause of mortality, especially in premature infants. One of the factors that contribute to the susceptibility of these patients to infections is the immaturity of their immune system. One of the possible genesis of this phenomenon seems to lie in the presence of an inhibitor of placental origin, which acts by decreasing the endogenous production of the granulocyte-colony stimulating factor (G-CSF). A systematic review was performed using the PICO search system. Newborns with neonatal sepsis and neutropenia (patient), Treatment with G-CSF plus antibiotics compared with conventional therapy (intervention), Death (any cause), and adverse effects (outcome). The search resulted in 85 articles, of which 12 studies were included. The details of the methodology and results of this guideline are set out in Annex 1.

## INTRODUCTION

Sepsis is a serious complication in newborns and an important cause of mortality, especially in premature infants[1,2]. Although there has been a reduction in its incidence in recent decades[3], it is estimated that 1.4 million newborns still die annually as a result of invasive infection[4].

In addition to mortality, septic conditions are associated with a worse neurological prognosis and the occurrence of chronic pulmonary disease[5,6], impairing the quality of life and raising the social costs of survivors.

One of the factors that contribute to the susceptibility of these patients to infections is the immaturity of their immune system[4]. Structural and functional abnormalities of the immune components have been described in this age group, and the changes probably more related to the occurrence of sepsis are those of

polymorphonuclear cells[7]. Among them, neutrophils also presented a quantitative reduction in up to 58% of preterm infants, depending on the definition of neutropenia used[8].

Neutrophils play a fundamental role in the defense against bacterial infections, and people with a chronic deficiency of these cells exhibit recurrent infections and early mortality. In neonates, risk factors such as gestational hypertension, intrauterine growth restriction, fetus-fetal transfusion syndrome, and hemolytic anemia due to Rh incompatibility are related to the absolute reduction in the count of these cells[9].

One of the possible genesis of this phenomenon seems to lie in the presence of an inhibitor of placental origin, which acts by decreasing the endogenous production of the granulocyte-colony stimulating factor (G-CSF)[10]. In addition, it has been observed that newborns with a low number of neutrophils in the bloodstream, in fact, have a hidden a medullary reserve, which can quickly respond to the use of a recombinant stimulating factor (rG-CSF)[11].

For this reason, rG-CSF has been suggested as prevention and adjuvant treatment of infections in the neonatal period[12]. Clinical trials and systematic reviews concluded that the drug is safe for this population, but were not sufficient to recommend its routine use[13].

## RESULTS

The population included 355 newborns with sepsis and undergoing treatment with G-CSF plus antibiotics (N=183), compared (N=172) with conventional therapy (N=179), and followed-up to measure the death outcomes due to all causes related (in time) to the septic episode during hospitalization and death at 14 or 28 days, in addition to adverse events (Table 1).

In order to achieve some comparability between studies, the data for the death outcome were recovered in such a way as to allow an analysis of mortality at the 14th or 28th day, from the beginning G-CSF therapy.

Among the studies selected, the mean gestational age of the patients ranged from 24 to 40 weeks (only one study included NB <40 weeks[24]; seven studies[17-23] included NB <37 weeks). The body weight ranged from 500 to 3,667g, and age was ≤28 days. The median duration of G-CSF treatment was four days (range: 3-14 days) with a dose of 10 mcg/kg/

**TABLE 1.** SEPSIS IN NEWBORNS WITH NEUTROPENIA ≤5,000 CELLS/MM³

| DESCRIPTION OF THE STUDIES INCLUDED | | | | |
|---|---|---|---|---|
| Study | Population | Intervention | Control | Outcome/ Time |
| Bedford-Russell, 2001<br>Multicenter | 28 NB with clinical signs of sepsis and neutrophils <5 x 10(9)/L.<br>IG >25s, PC 500 – 1,500g, Id ≤28 d | 13 NB, G-CSF 10 mcg/kg/d, IV, maximum of 14 d | 15 NB, placebo | Death during hospitalization<br>Death at 14 days<br>Death at 28 days |
| Drossou-Agakidou, 1998<br>Single Center | 35 NB with clinical signs of sepsis and neutrophils <5 x 10(9)/L.<br>IG 24 – 37s, PC 720 – 2,940 g, Id <28 d | 19 NB, G-CSF 10 mcg/kg/d, SC, 3 d | 16 NB, placebo | Death at 14 days |
| Schibler, 1998<br>Multicenter | 20 NB with early-onset sepsis and neutropenia <1.7x10⁹/L.<br>IG 24 – 40s, PC 530 – 3,667 g, Id <3 d | 10 NB, G-CSF 10 mcg/kg/d, IV, 3 d | 10 NB, placebo | Death during hospitalization<br>Death at 14 days<br>Death at 28 days |
| Gathwala, 2011<br>Single Center | 40 NB with clinical signs of sepsis and neutrophils <5 x 10(9)/L.<br>IG <37 s, PC <2,000 g | 20 NB, G-CSF 10 mcg/kg/d, IV, 5 d | 20 NB, conventional therapy | Death during hospitalization |
| Chaudhuri, 2012<br>Single Center | 78 NB with early-onset sepsis, neutropenia <1,500 cells/mm³<br>IG <34 s; PC 1,100 to 2,500 g; Id 3 d | 39 NB, G-CSF 10 mcg/kg/d, 3 d | 39 NB, placebo | Death during hospitalization |
| Borjianyazdi, 2013<br>Single Center | 46 NB with clinical signs of sepsis; neutropenia (CAN ≤5,000/µL)<br>IG 30 – 37 s, PC 530 – 3,667 g, Id <10 d | 23 NB, G-CSF 10 mcg/kg/d, SC, 5 d | 23 NB, placebo | Death at 14 days |
| Aktaş, 2015<br>Single Center | 56 NB with sepsis, neutropenic (<1.0 x 10⁹/L)<br>IG 32 s, PC 1,001 ± 240 | 33 NV, G-CSF 10 mcg/kg/d, IV, (1– 4 d; median, 2 d) up to CAN ≥1.0 x 10⁹/L | 23 NB, conventional therapy | Death during hospitalization |
| Gupta, 2016<br>Single Center | 52 NB with clinical signs of sepsis, neutropenia <1,800 cells/mm³<br>IG 32 s, PC 1500.38 ±306.35, Id <25 d | 26 NB, G-CSF 10 mg/kg/d, 3 d, SC | 26 NB, conventional therapy | Death during hospitalization |

NB = newborns, CAN = absolute neutrophil count, IG = gestational age, s = weeks, PC = body weight, Id = age, d = days, IV = intravenous, SC = subcutaneous

**TABLE 2.** DESCRIPTION OF THE BIASES OF THE STUDIES INCLUDED

| Study/Year | Rand | Blinded allocation | Blinded partici-pants and team | Blinded evaluator | Losses | Prog. character-istics | Appro-priate outcomes | AITT | Sample size calcu-lation |
|---|---|---|---|---|---|---|---|---|---|
| Aktas 2015 | | | | | | | | | |
| Bedford-Russel 2001 | | | | | | | | | |
| Borjianyazdi 2013 | | | | | | | | | |
| Chaudihuri 2012 | | | | | | | | | |
| Drossou-Agakidou 1998 | | | | | | | | | |
| Gathwala 2011 | | | | | | | | | |
| Gupta 2016 | | | | | | | | | |
| Schibler 1998 | | | | | | | | | |

ITTA = Intention to Treat Analysis; Boxes: green = absence of bias, red = presence of bias, yellow = uncertain risk of bias

day, intravenously, or subcutaneously. Four studies[20,23,24,17] included NB with a cutoff point for neutropenia <1,800 cells/mm$^3$, and four others[18,19,21,22] with neutropenia ≤5,000 cells/mm$^3$.

In relation to the risk of bias, there was no description of the randomization in two studies[17.21] and of the blinded allocation in three[17,21,24]. No blinding of the team was described in one study[17,] and there was no double-blinding in another[21]. One study presented a loss of ≥20%, and no intention to treat analysis was conducted[17]. The overall risk of bias of the studies included is considered not severe (Table 2).

## META-ANALYSIS FOR THE DEATH OUTCOME

The meta-analyzed outcomes were death during hospitalization and death at 14 or 28 days from the beginning of the G-CSF therapy.

In the analysis of in-hospital mortality, there was a reduction in the risk by 19% (95% CI, 10 to 29; $I^2$=26%) with the use of G-CSF in comparison to the conventional treatment with or without placebo; it was necessary to treat about 5 NB to prevent one death (NNT). The quality of evidence is high to sustain this outcome (Table 3).

In the analyses of mortality at 14 or 28 days, there was no difference in the use of G-CSF in comparison to the conventional treatment, with or without a placebo. The quality of evidence is moderate to sustain this outcome (Table 3- Annexes).

### Heterogeneity

There was no evidence of significant heterogeneity between the studies.

### Adverse effects of G-CSF administered in newborn sepsis

No serious adverse effects were reported with the use of G-CSF in the studies evaluated in this review. There were mild adverse effects, such as cutaneous reactions, irritability, electrolytic alterations, cardiac alterations, peripheral edema, and thrombocytopenia, but with no significant difference between G-CSF and the placebo. Four of the eight studies reported no adverse effects[19-22].

## SYNTHESIS OF EVIDENCE

In newborns with sepsis and neutropenia ≤5,000 cells/mm$^3$, the use of G-CSF reduces the risk of in-hospital death by 19% (NNT = 5). G-CSF has proved to be a safe drug.

## DISCUSSION

In a previous meta-analysis conducted by Bernstein (2001), five studies with a total of 155 patients were evaluated, and the mortality rate was lower among patients who received G-CSF than among those who received the placebo. However, when the non-randomized studies were excluded, the beneficial effects of

**TABLE 3.** G-CSF COMPARED TO THE CONVENTIONAL TREATMENT IN NEONATAL SEPSIS WITH NEUTROPENIA (CAN <5,000 CELLS/MM³)

| Evaluation of certainty | | | | | | | Summary of Results | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| No. of Participants (studies) Follow-up | Risk of bias | Inconsistency | Indirect evidence | Imprecision | Publication bias | Overall certainty of the evidence | Study event rates (%) | | Relative Effect (95% CI) | Potential absolute effects | |
| | | | | | | | With control | With G-CSF | | Risk with control | Risk difference with G-CSF |
| DEATH (HOSPITALIZATION) | | | | | | | | | | | |
| 274 (6 RCTs) | not severe | not severe | not severe | not severe | None | ⊕⊕⊕⊕ HIGH | 46/133 (34.6%) | 22/141 (15.6%) | RR 0.43 (0.27 to 0.69) | 346 per 1,000 | minus 197 per 1,000 (from minus 252 to minus 107) |
| DEATH (14 DAYS) | | | | | | | | | | | |
| 129 (4 RCTs) | not severe | not severe | not severe | severe [a] | none | ⊕⊕⊕○ MODERATE | 10/64 (15.6%) | 8/65 (12.3%) | RR 0.79 (0.34 to 1.81) | 156 per 1,000 | 33 less per 1,000 (from 103 less to 127 more) |
| DEATH (28 DAYS) | | | | | | | | | | | |
| 48 (2 RCTs) | not severe | not severe | not severe | severe [a] | none | ⊕⊕⊕○ MODERATE | 7/25 (28.0%) | 3/23 (13.0%) | RR 0.46 (0.13 to 1.57) | 280 per 1,000 | 151 less per 1,000 (from 244 less to 160 more) |

CI: Confidence interval; RR: Risk ratio. Explanations = [a]. Non-significant difference.

**FIGURE 2.**- DEATH DURING HOSPITALIZATION. COMPARISON CHART: G-CSF *VERSUS* CONVENTIONAL THERAPY, OUTCOME: ALL-CAUSE MORTALITY DURING HOSPITALIZATION.

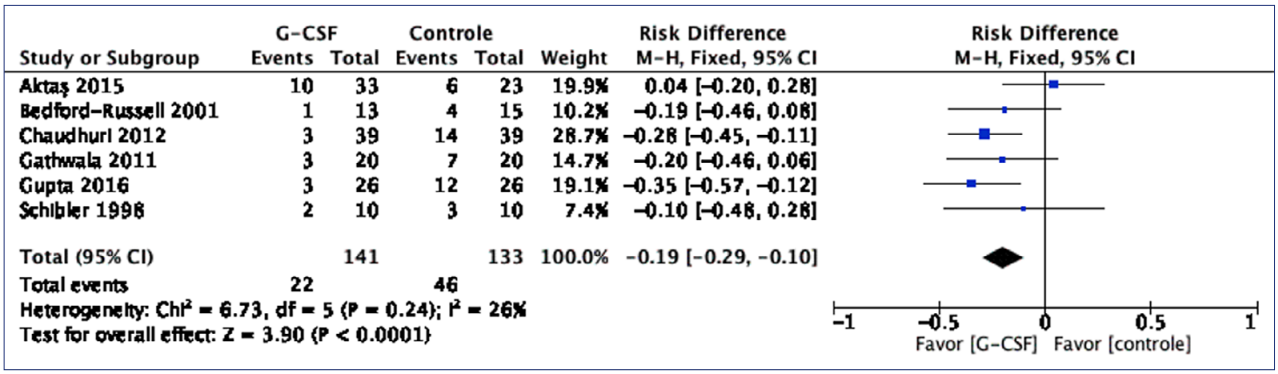



**FIGURE 3.** DEATH AT 14 OR 28 DAYS FROM THE BEGINNING OF THE G-CSF THERAPY. COMPARISON CHART: RHG-CSF *VERSUS* CONVENTIONAL THERAPY, OUTCOME: MORTALITY DUE TO ALL CAUSES: 14 DAYS

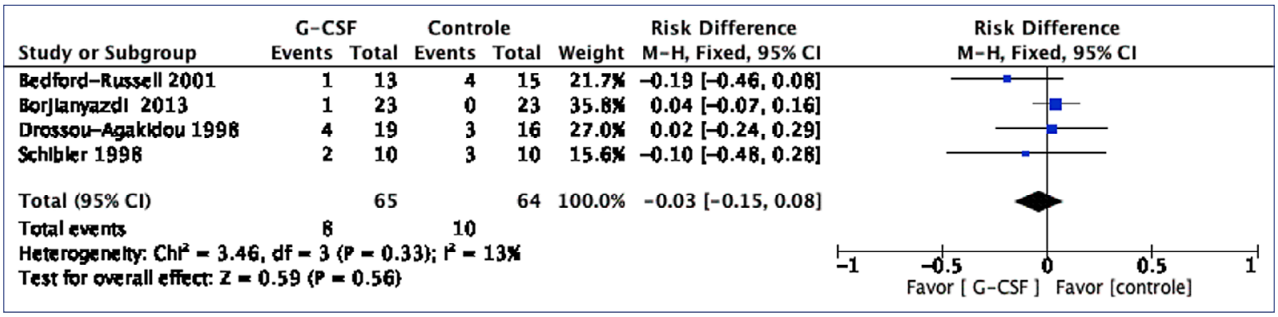
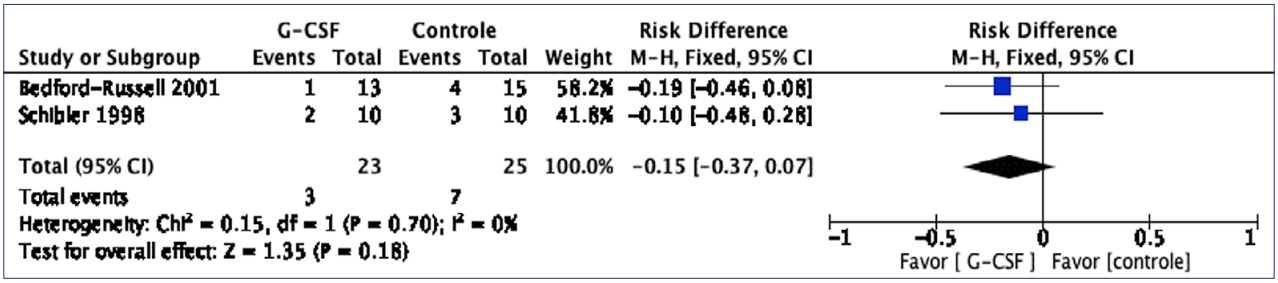



**FIGURE 4.** COMPARISON CHART: RHG-CSF *VERSUS* CONVENTIONAL THERAPY, OUTCOME: MORTALITY DUE TO ALL CAUSES AT 28 DAYS.



G-CSF therapy were found to be less consistent. Thus, the routine use of G-CSF could not be recommended for all newborns with sepsis[25].

In the Cochrane Systematic Review, Carr and Modi (2003) published a meta-analysis of seven studies to determine the safety and efficacy of rhG-CSF to reduce mortality in the treatment of suspected or proven systemic infections. They concluded there was no evidence to support the addition of G-CSF or granulocyte-macrophage colony-stimulating factor (GM-CSF) to antibiotic therapy in preterm infants with suspected systemic infection to reduce mortality. A subgroup analysis of 97 infants of three treatment studies which, in addition to systemic infection, presented clinically significant neutropenia (<1.7, 109/L) at the time they were enrolled in the study shows a significant reduction in mortality until the 14th day [RR, 0.34 (95% CI, 0.12, 0.92); RD, 0.18 (95% CI, 0.33, 0.03); number needed to treat, 6 (95% CI, 3,33)][26].

In this systematic review with meta-analysis, we included eight randomized clinical trials on G-CSF therapies in newborns with sepsis and neutropenia ≤5,000 cells/mm³ comparing them with the conventional treatment, and that reported all-cause mortality. The review showed evidence, based on data from six studies included in the meta-analysis, that the addition of G-CSF to antibiotic therapy in newborns with sepsis and neutropenia <5,000 cells/mm³ reduces mortality in hospitalization due to all causes [RD -0.19 (95% CI -0.29, 0.10); number needed to treat, 5 (95% CI, 3, 11)].

No significant advantage in survival was observed at 14 and 28 days from the beginning of therapy [RD -0.03 (95% CI -0.15, 0.08) and RD -0.15 (95% CI -0.37, 0.07), respectively]. However, all eight treatment studies were small, with the largest including only 78 newborns.

Our study has some limitations. The studies included in our meta-analysis were performed in a wide range of time, during which the definition of neonatal sepsis and the diagnostic and treatment methods changed. Four different definitions of neutropenia were used in different studies, and the definitions of sepsis varied between the clinic one and proof through positive culture. Patients of multiple birth weights and gestational ages were reported in all eight studies, and it is possible that this is a significant confounding factor.

## ANNEX I

### Clinical question

What is the impact on overall mortality outcomes (death from any cause) and adverse events of G-CSF in the treatment of newborns with neonatal sepsis and neutropenia [Absolute Neutrophil Count (ANC)<5,000 mm³] compared to the conventional therapy?

### Objective

The objective of this assessment is to identify the efficacy and safety of granulocyte-colony stimulating factor (G-CSF) in the treatment of neonatal sepsis with neutropenia less than 5,000 cells/mm³, in comparison with conventional therapy.

### Structured question

| | |
|---|---|
| **P** | Newborns with neonatal sepsis and neutropenia (CAN ≤5,000 cells/mm³) |
| **I** | Treatment with G-CSF plus antibiotics compared with the conventional therapy |
| **C** | - |
| **O** | Death (due to any cause) and adverse effects |

### Eligibility criteria

We followed the recommendations of the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (Prism) to conduct this systematic review[14].

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

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

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

Excluded outcome - failure in the correction of preexisting neutropenia during treatment.

Randomized clinical trial studies were selected.

Without time or language restrictions.

Full text available for access.

### Search for papers

Databases and research strategy

The search for evidence will be conducted on virtual scientific information databases, using the following search strategy:

- Medline/PubMed - (Granulocyte Colony-Stimulating Factor OR rG-CSF OR G-CSF OR GCSF OR

Recombinant Proteins) AND (Infant, Newborn OR Infant, Premature OR neonates OR preterm infants) AND (Sepsis OR Neutropenia) AND Random*.

- Central/Cochrane Library - (Granulocyte Colony-Stimulating Factor OR rG-CSF OR G-CSF OR GCSF OR Recombinant Proteins) AND (Infant, Newborn OR Infant, Premature OR neonates OR preterm infants) AND (Sepsis OR Neutropenia).

- Lilacs via BVS - same search as in Central/Cochrane Library.

We also searched sources of data still unpublished, in progress, in gray literature and manual, performed by checking the list of "References" of the studies included in this review or in previous reviews.

All searches were performed by October 2019.

## CRITICAL EVALUATION

### Relevance - clinical importance

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

### Results application - External validity

The process of retrieving the studies, as well as the evaluation of the titles and abstracts obtained, will be conducted by two researchers with expertise in the development of systematic reviews (A. S. and W. M. B.), independently and blinded, in accordance with the eligibility criteria listed above. When there is any disagreement regarding the selection of studies between the researchers, a third reviewer will be consulted (I. F.).

## RESULTS

The data extraction will be performed independently by three reviewers, the results compared, and disagreements resolved by discussing them.

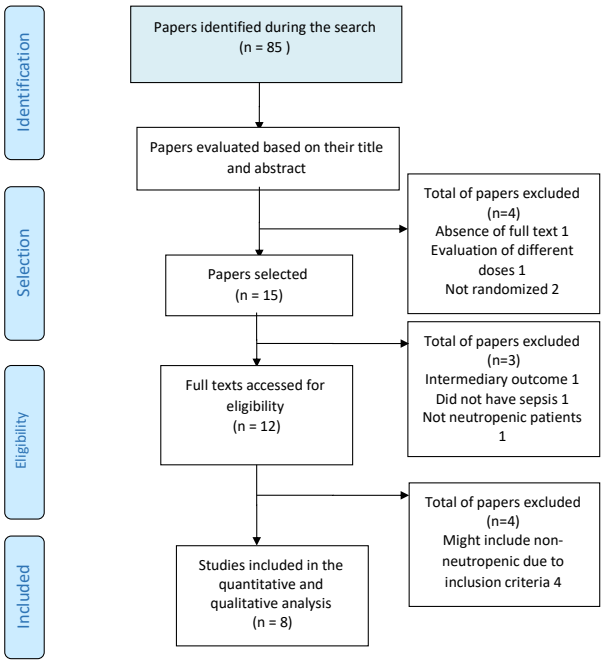
We will extract the following data from the studies: name of the author and year of publication, study population, intervention and comparison methods, the absolute number of deaths and adverse events, time of follow-up.

The methodological quality of the eligible trials will be evaluated independently by individual reviewers, not blinded, using the full text of the paper. Randomized clinical trials will have their risk of biases analyzed according to the following criteria: randomization, blinded allocation, double-blinding, losses, prognostic characteristics, presence of relevant outcome, time for the outcome, the method for outcome measurement, sample size calculation, early interruption, presence of other biases.

The results of the studies included will be meta-analyzed using the RevMan 5.3 software [15]. The heterogeneity of treatment effects between the tests was evaluated graphically and tested using the standard chi-square test. A weighted estimate of the relative risk (RR) between the studies was calculated using a fixed-effects model. The risk difference (RD) and the number needed to treat (NNT) were also calculated, and the final measure was used to sustain the synthesis of evidence, which will answer the clinical question of this review. The 95% CI was calculated for all outcomes.

During the search for evidence, we retrieved 85 papers, of which 12 were then selected based on their title and abstract; they all evaluate the use of G-CSF plus antibiotics for the treatment of newborns with sepsis and neutropenia, in comparison with conventional therapy. The 12 studies that met the eligibility criteria were then were accessed for analysis of their full text. Of the 12 studies, five[17-24] were selected to support this review; the grounds for exclusion and the list of studies excluded are available in the references and in Figure 1.

**FIGURE 1.** THE SELECTION OF RETRIEVED FROM THE VIRTUAL DATABASES OF SCIENTIFIC INFORMATION



## Application of evidence - Recommendation

The quality of evidence will be graded as high, moderate, low, or very low using the Grade instrument[16] and taking into account the risk of bias, the presence of inconsistency, vagueness or indirect evidence in the meta-analysis of the outcomes of death and adverse events, and the presence of publication bias.

## Conflict of interest

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

## Final declaration

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

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MIGHT HAVE INCLUDED NON NEUTROPENIC

# Depression in the workplace: screening and treatment

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**Question: What evaluation is needed for better diagnosis and planning of patients with complex renal lithiasis who must be submitted to surgical treatment, such as percutaneous kidney lithotripsy?**

**Answer:** Screening for depression in workers is recommended because of its high prevalence and underdiagnoses in the workplace. There is evidence that depression in workers has a relevant impact on occupational indicators and on the generation of comorbidities. Therefore, its early diagnosis and identification is recommended, as well as specific interventions, including actions on risk factors for depression at work. Thus, screening for depression needs to be followed by diagnostic confirmation and pharmacological and non-pharmacological therapeutic measures, with the benefit and safety being verified in occupational outcomes such as presenteeism, absenteeism, prolonged leaves, work accident, commute accident, activity restriction, perception of health and adverse events for patients.

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# Flucloxacillin-Induced Hepatotoxicity - Association with HLA-B*5701

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## SUMMARY

*Drug-induced liver injury (DILI) to flucloxacillin is rare and is classified as idiosyncratic, as it is dependent on individual susceptibility, unpredictable, and dose-independent.*

*The authors present the case of a 74 - year - old man with a history of monoclonal gammopathy under investigation and alcoholic habits of 24 g/day, with asthenia, anorexia, nausea, abdominal discomfort, and fever with three days of evolution. He was treated with two courses of antibiotic therapy with flucloxacillin to erysipelas previously (3 months and 2 weeks before admission). Lab tests showed serum AST levels of 349 U/L, ALT 646 U/L, alkaline phosphatase 302 U/L, GGT 652 U/L, total bilirubin 3.3 mg/dL and direct bilirubin 2.72 mg/dL. Infectious, autoimmune, and metabolic causes were ruled out. Magnetic resonance cholangiopancreatography showed normal results. Liver biopsy showed mild multifocal (predominantly microvesicular) steatosis; marked changes in the centrilobular areas (sinusoidal dilatation, marked congestion, hemorrhage, and multifocal hepatocyte collapse); expansion of the portal areas with the formation of bridges; proliferated bile ducts and inflammatory infiltrate of variable density, predominantly mononuclear type. The HLA-B*5701 screening test was positive.*

*Hepatic biochemical tests remain abnormal with a significative increase in total bilirubin, which reached levels of 24.1 mg/dL, with the development of jaundice, pruritus, and choluria. DILI was assumed, and the patient was treated with ursodeoxycholic acid. There was favorable evolution, without evidence of blood coagulation dysfunction or encephalopathy. The analytic normalization was, however, slow, with evolution to chronicity.*

*The authors present this case to remind the possibility of moderate/severe drug-induced liver injury to flucloxacillin, an antibiotic commonly used in clinical practice and association with the HLA-B * 5701 allele reported in the literature.*

KEYWORDS: *Chemical and Drug-Induced Liver Injury. Floxacillin. HLA-B Antigens.*

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## INTRODUCTION

Drug-associated hepatotoxicity is a challenge in current clinical practice, either by its reduced incidence or by the absence of specific biomarkers. The recognition and diagnosis are potentially complex due to the similarity of the clinical, laboratory, and histological presentation with other causes of liver disease.[1,2]

The diagnosis is clinical, based on exclusion, and assumed, since the test of higher predictive value would be the reexposure to the drug, which is contraindicated in most cases.[3]

Hepatotoxicity is a known adverse effect, however unusual, of flucloxacillin. Its prevalence in the first exposure has been estimated at 1/10,000, and most cases occur after three months. Hepatotoxicity induced by flucloxacillin is a B-type or idiosyncratic reaction.[4] This type of reaction occurs in an unpredictable way, often regardless of the dose, and usually at therapeutic levels. It presents a low incidence, a phase of variable latency, and there are usually immune mechanisms involved. The anatomopathological lesions are uncharacteristic and uneven. Systemic manifestations, such as fever, rash, arthralgia, eosinophilia, leukocytosis, and appearance of autoantibodies are frequent.[2,3] Due to its rarity and unpredictability, these are rarely detected in pre-clinical or clinical trials of drugs. In addition, its reduced incidence makes it even more difficult to investigate it in epidemiological studies.[4]

The most common risk factors for flucloxacillin-related hepatotoxicity are the female gender, age above 60 years, and duration of treatment exceeding two weeks.[4] The HLA system plays a key role in immune-mediated adverse reactions to drugs. The HLA-B*5701 allele is common in northern Europe, and it is estimated that it occurs in 6-8% of the general European population. Studies show that 84.3% of patients with hepatitis due to flucloxacillin have the HLA-B*5701 and 80 times greater risk of being affected.[5] HLA typing can substantiate the diagnosis of hepatotoxicity due to specific drugs; however, its validation is required before recommending routine implementation, since it is currently not cost-effective.[2]

The diagnosis of hepatotoxicity depends on a high index of suspicion and is based on a compatible temporal relationship, a suggestive analytical pattern of liver enzymology, improvement after suspension of the drug (with known toxicity), concomitant manifestations of hypersensitivity, exclusion of other etiologies, and application of diagnosis scales.[6] Lymphocyte transformation tests may also be useful.[3] A liver biopsy can substantiate the clinical suspicion of hepatotoxicity, provide important information about its severity, and help exclude other potential causes of liver injury;[7] however, there are no histological characteristics that unequivocally confirm the diagnosis.[7,8]

## CLINICAL CASE

A man, 74 years old, self-employed, metallurgical worker, attended the emergency service due to asthenia, anorexia, nausea, abdominal discomfort, and mild fever (maximum axillary temperature of 38.1 ºC) with three days of evolution. There was a reference to two previous eight-day cycles of antibiotic therapy with flucloxacillin 500 mg 8/8h due to erysipelas (three months and two weeks prior to admission). He had a history of monoclonal gammopathy under investigation, previous brucellosis for nearly 30 years, benign prostate hypertrophy treated with transurethral resection, and left inguinal hernioplasty. There was no usual chronic medication. Alcohol habit: 24 g/day. There were no other noteworthy habits.

Upon objective examination, the patient was hemodynamically stable, feverless, with hydrated and rosy mucous membranes, subicteric. His abdomen was soft, without palpable masses or enlarged organs, with slight discomfort upon general palpation, without defense or signs of peritoneal irritation, with air-fluid noises of normal timbre. There was no evidence of chronic liver disease.

Analysis showed hemoglobin 15.0 g/dL, VGM 92.6 fL, leucocytes 5.6x $10^9$/L, platelets 185 x $10^9$/L, AST 349 U/L (N: 4-43), ALT 646 U/L (N: 4-43), FA 302 (N: 25-100) U/L, GGT 652 U/L (N: 7.0-49.0), total bilirubin 3.3 mg/dL(N:0.3-1.2), direct bilirubin 2.72 mg/dL (N: 0.10-0.50), normal LDH, amylase, and lipase, normal coagulation, and albumin 4.0 g/dL. Renal function and ionogram without alterations, CRP 3.96 mg/dL (N<0.50) (Table 1). An abdominal ultrasound showed liver, pancreas, and spleen without evidence of lesions of acute nature; the gallbladder was a bit distended, without other evident changes.

The patient was admitted for investigation and monitoring. An additional etiologic study found ferritin 642.0 ng/mL(N: 22.0- 322.0) with normal remaining iron kinetics. Serum protein electrophoresis had a

Gama monoclonal peak, and immunoelectrophoresis of serum proteins showed the presence of antiserum lambda monoclonal IgG bands. Ceruloplasmin; normal serum and urinary copper and alpha-1 antitrypsin. Autoimmunity assay and hepatotropic viruses were negative.

A cholangial MRI was performed and showed the liver with normal dimensions and regular contours,

**TABLE 1.** ANALYTICAL INVESTIGATION OF THE PATIENT.

| Analysis | Results | Reference values |
|---|---|---|
| Complete blood count | | |
| Leukocytes<br>Neutrophils<br>Lymphocytes<br>Monocytes<br>Eosinophils<br>Basophils | 5.6 x$10^9$/L<br>2.4 x$10^9$/L (42.8%)<br>1.5 x$10^9$/L (27.0%)<br>0.9 x$10^9$/L (16.7%)<br>0.7 x$10^9$/L (13.0%)<br>0.0 x$10^9$/L (0.5%) | 4.5 - 11.5 |
| Hemoglobin | 15.0 g/dL | 14.0 - 18.0 |
| Platelets | 185 x$10^9$/L | 150.0 - 450.0 |
| Coagulation and Hemostasis | | |
| Prothrombin index | 89 % | 70.0 - 100.0 |
| INR | 1.06 | |
| APTT | 28.8 seconds | 21.9- 32.9 |
| Biochemistry | | |
| Albumin | 4.0 g/dL | 3.5 - 5.0 |
| Amylase | 20 UI/L | 8-53 |
| Lipase | 22 UI/L | 6-51 |
| LDH | 551 UI/L | 200 - 480 |
| CK | 101 UI/L | 0 - 165 |
| CRP | 3.96 mg/dL | <0.50 |
| Procalcitonin | 0.29 ng/mL | 0.01-0.64 |
| Iron | 140.1 µg/dL | 45.0 - 182.0 |
| Ferritin | 642.0 ng/mL | 22.0 - 322.0 |
| Total capacity of iron binding | 286.2 µg/dL | 258.9-388.0 |
| Transferrin | 219.5 mg/dL | 180.0-380.0 |
| Ceruloplasmin | 40.30 mg/dL | 22.0 - 58.0 |
| Alpha-1 antitrypsin | 172.0 mg/dL | 80.0 - 199.0 |
| Urinary copper | 38 µg/L | 9-62 |
| Infectious Serologies | | |
| CMV, EBV, Hepatitis B and C, Syphilis, HIV, Brucellosis, Leptospira, Borrelia, Coxiella | Negative for acute infection | |
| Immunoelectrophoresis | | |
| Serum IgG | 2228.0 mg/dL | 650.0- 1600.0 |
| Serum IgA | 138.0 mg/dL | 40.0- 350.0 |
| Serum IgM | 39.0 mg/dL | 50.0- 300.0 |
| Serum Kappa light chains | 389.0 mg/dL | 598.0-1329.0 |
| Serum lambda light chains | 1170.0 mg/dL | 280.0-665.0 |
| Serum IgG antiserum and lambda | Presence of monoclonal bands | |
| Urinary immunoelectrophoresis | No changes | |
| Endocrinology | | |
| TSH | 0.157 mUI/L | 0.350 - 5.500 |
| T3L | 2.9 pg/mL | 2.0 - 4.2 |
| T4L | 1.2 pg/mL | 0.9 - 1.8 |
| Ac- Anti-TSH receptor | Negative | |
| Immunology | | |
| ANA, Ac anti-DNA, ENA screen, antimitochodria, anti-M2, anti- SP100, anti-smooth muscle, anti-factin, anti-SLA/LP, anti-LKM1, anti-LC1, anti-MPO, and anti-PR3 | Negative | |

smooth texture, without focal lesions. There were no pathologies of the biliary tract. Bone marrow biopsy and examination were consistent with monoclonal gammopathy of undetermined significance.

During hospitalization, the patient remained feverless, hemodynamically stable, without abdominal pain, and analytically without elevation of parameters that were inflammatory or suggestive of an infectious process. We observed decreasing profile values of AST, ALT, GGT, and AP (although the latter with variations) (Fig. 1); however, there was progressive worsening of the values of bilirubin, reaching 24.1 mg/dL for total bilirubin and 22.32 mg/dL for direct, with development of jaundice, pruritus, and choluria, without coagulopathy or encephalopathy.

Based on the application of diagnosis scales, the sum of the scores from the Maria and Vitorino[i9] scale is 13 points (possible causality ), and from the Rucam[10] scale is 8 points (likely causality). Once hepatotoxicity was confirmed, in this follow-up, treatment with ursodeoxycholic acid was started at 1,500 mg/day, in the 21st day of hospitalization.

The liver biopsy later revealed slight multifocal steatosis (predominantly microvesicular); accentuated changes in the centrilobular areas (sinusoidal dilatation, marked congestion, hemorrhage, and multifocal collapse of hepatocytes); expansion of the portal areas with development of bridges; proliferated bile ducts and inflammatory infiltrate of variable density, predominantly of the mononuclear type. The test for amyloid substance using Congo red staining was negative. HLA-B*5701 typing was performed with positive results.

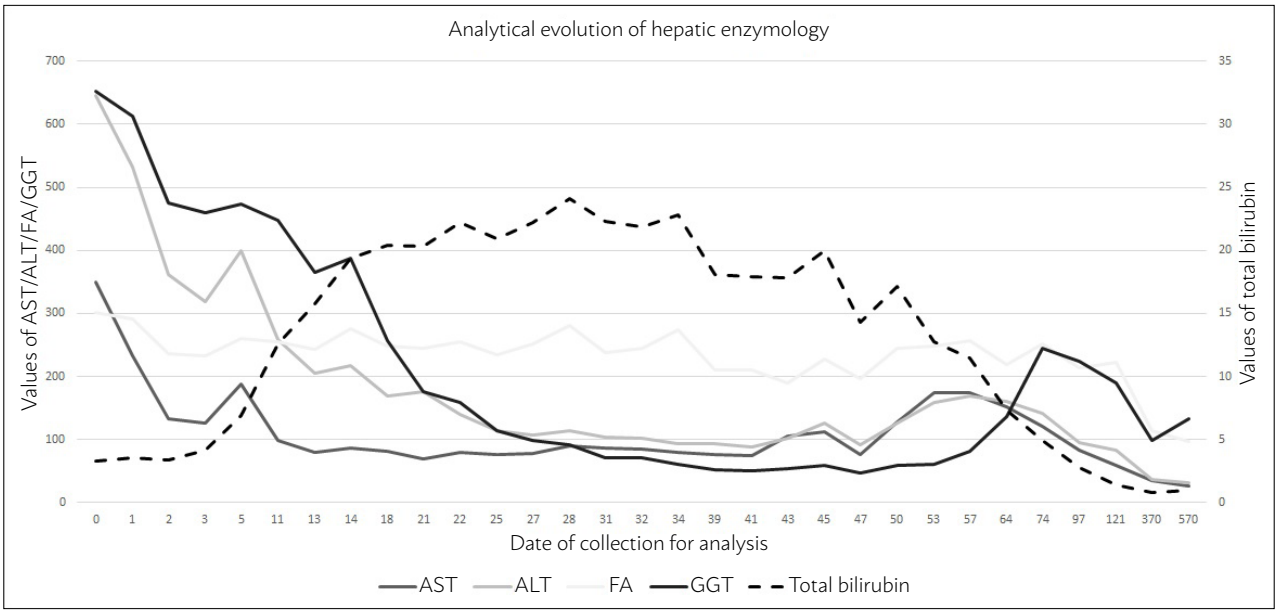
After flucloxacillin had been suspended for four months, there was clinical and analytical improvement with AST 60 U/L, ALT 83 U/L, FA 222 U/L, GGT 190.3 U/L, total bilirubin 1.4 mg/dL and direct bilirubin 1.06 mg/dL. About a year later, there was still a slight elevation of FA (114 U/L) and GGT 99.5 U/L with the other liver enzymology figures within the normal range; after 18 months, only GGT remained high.

## DISCUSSION

The clinical case in question presents a case of mixed hepatotoxicity — R-value of 4.97 [R=(ALT/upper limit of normality)/(FA/upper limit of normality)].[2,6] It is recognized that the period between the first dose of flucloxacillin and the beginning of laboratory manifestations was approximately three months and two weeks after re-exposure to the drug, possibly having started earlier, although it is not possible to affirm it objectively. The age of the patient, the concomitance of monoclonal gammopathy under investigation, and exuberance of analytical raised the hypothesis of infiltrative liver disease, in particular, due to amyloidosis, which, similarly to other possible etiologies, was excluded. Then, mild fever and eosinophilia were considered extra-hepatic manifestations of hepatotoxicity.

With respect to the analytical developments (Fig. 1), we could initially verify an analytical pattern of hepatotoxicity even more suggestive of hepatocellular

**FIGURE 1.**



and, at a later stage later, compatible with a cholestatic pattern. It should be noted that the biochemical categorization of the hepatotoxicity pattern is not always indicative of the underlying pathological pattern.[7,8]

According to the classifications of hepatotoxicity severity, this case can be classified as moderate (International Dili Expert Working Group) or moderate-severe (US Drug-Induced Liver Injury Network).[2]

The anatomopathological findings showed signs that may be associated with a worse prognosis, with the need for liver transplantation and higher mortality, namely the presence of microvesicular steatosis, hepatocyte necrosis, cholangial cholestasis, and duct proliferation.[2]

In the absence of an antidote, the basis for the treatment of toxic hepatitis is based on the suspension of the drug and therapeutic support. In patients with evidence of hepatic cholestasis, ursodeoxycholic acid may be a therapeutic alternative, although its effectiveness is limited, and its use is controversial.[2,11] In the presence of hepatotoxicity associated with systemic hypersensitivity and characteristics compatible with autoimmune hepatitis, corticosteroid therapy can be considered, although the evidence is also limited.[2,11]

The presence of jaundice concomitant to the hepatotoxicity scenario is associated with approximately 10% likelihood of risk of progression to fatal liver failure. Generally, the hepatocellular pattern is associated with a worse prognosis and high mortality. The cholestatic pattern can also be associated with significant mortality, while the mixed pattern seems to be associated with a lower risk.[2]

## CONCLUSION

The authors present a case of mixed hepatotoxicity due to flucloxacillin that caused prolonged hospitalization and comprehensive etiological study. Despite the moderate to severe analytical presentation and the histological findings indicative of poor prognosis, there was favorable evolution, without clinical evidence of coagulopathy or encephalopathy. The analytical normalization was, however, slow, with progression to chronicity. Studies associating the HLA-B*5701 allele with susceptibility to toxicity by flucloxacillin have been reported in the literature. Although this is still not a cost-effective screening, it allows identifying susceptible individuals, preventing exposure to the drug, or indicating the need for closer monitoring. Lastly, it allows substantiating the diagnosis.

### Contribution of the authors

Monica Teixeira was responsible for interpreting the case, designing, and writing the article. Sara Macedo contributed to designing and writing the article. Tânia Batista contributed to designing and writing the article. Sofia Martins contributed to interpreting the case and writing the article. Andreia Correia contributed by critically reviewing the article. Luis Costa Matos contributed by critically reviewing the article.

### Conflicts of Interest

The authors declare there are no conflicts of interest associated with this publication.


## RESUMO

*A hepatotoxicidade à flucloxacilina é rara e classifica-se como idiossincrática, uma vez que é dependente da suscetibilidade individual, não expectável e independente da dose.*

*Apresentamos o caso de um homem, 74 anos, antecedentes de gamapatia monoclonal e hábitos alcoólicos de 24 g/dia, com quadro de astenia, anorexia, náuseas, desconforto abdominal e febrícula com três dias de evolução. Referência a dois ciclos de antibioterapia com flucloxacilina por erisipela (três meses e duas semanas antes da admissão). Analiticamente com AST 349 U/L, ALT 646 U/L, FA 302 U/L, GGT 652 U/L, bilirrubina total 3,3 mg/dL, bilirrubina direta 2,72 mg/dL. Excluídas etiologias infecciosa, autoimune, metabólica, bem como patologia das vias biliares por colangio-RM. Biópsia hepática mostrou esteatose multifocal ligeira (predominantemente microvesicular); alterações acentuadas nas áreas centrolobulares (dilatação sinusoidal, congestão acentuada, hemorragia e colapso multifocal de hepatócitos); expansão das áreas portais com constituição de pontes; ductos biliares proliferados e infiltrado inflamatório de densidade variável, predominantemente de tipo mononucleado. Tipagem de HLA-B*5701 positiva. Agravamento analítico atingindo bilirrubina total 24,1 mg/dL, com desenvolvimento de icterícia, prurido e colúria. Admitida a hepatotoxicidade, iniciou terapêutica com ácido ursodesoxicólico. Verificou-se evolução favorável, sem evidência de coagulopatia ou encefalopatia. A normalização analítica foi, no entanto, lenta, com evolução para cronicidade.*

*Os autores apresentam este caso para alertar para a possibilidade de hepatotoxicidade moderada a grave à flucloxacilina, antibiótico de uso comum na prática clínica e associação com o alelo HLA-B*5701 relatada na literatura.*


PALAVRAS-CHAVE: *Doença hepática induzida por substâncias e drogas. Flucloxacilina. Antígenos HLA-B.*

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ORIGINAL ARTICLE

# Clinical effects of two combinations of olfactory agents on olfactory dysfunction after upper respiratory tract infection during olfactory training

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## SUMMARY

OBJECTIVE: *To compare two combinations of olfactory agents for olfactory training therapy of olfactory dysfunction after upper respiratory tract infection (URTI) and investigate the influencing factors on clinical effects.*

METHODS: *125 patients with olfactory dysfunction were randomly divided into two groups: test and control. During the olfactory training, four odors were used in both groups. The olfactory training lasted for 24 weeks. Then, participants were tested using Sniffin' Sticks and threshold-discrimination-identification (TDI) composite scoring before treatment and at 1, 3, and 6 months after treatment. The TDI scores were compared at different time points between the groups and within them, and influence factors were analyzed.*

RESULTS: *There was no significant difference in TDI scores between both groups. Furthermore, TDI scores did not significantly change after one month of treatment in either of the groups. After 3 and 6 months of treatment, TDI scores both significantly increased, and the odor discrimination and identification abilities significantly strengthened in both groups; however, the odor thresholds did not improve. The course of the disease was a significant influencing factor on the therapeutic effect of olfactory training for both groups.*

CONCLUSION: *The combination of essential balm, vinegar, alcohol, and rose perfume for olfactory training, which are scents commonly found in daily life, can effectively cure URTI-induced olfactory dysfunction, and significantly improve the odor discrimination and identification abilities. Furthermore, prolonging the treatment time can help with the recovery of olfactory functions, and earlier olfactory training can improve the therapeutic effect.*

KEYWORDS: *Olfaction Disorders. Olfactory Perception. Training. Smell/physiology. Infection.*

## INTRODUCTION

Olfactory dysfunction is a common symptom during otolaryngology outpatient service and is mainly induced by three causes, including upper respiratory tract infection (URTI), nose and sinus diseases, and head injuries. In particular, the incidence rate of secondary olfactory dysfunction after URTI is 37.9%.[1] During the clinical treatment of secondary olfactory dysfunction after URTI, and in addition to drug

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treatment, olfactory training has attracted growing attention in recent years. In this new therapy of olfactory training, the recovery of olfactory functions is promoted through the periodical and repeated active smelling of diverse everyday odors. Clinical studies have indicated that this training is beneficial for the olfactory functions in olfactory dysfunction patients.[2-5] The conventional olfactory agents used in olfactory training are mostly standardized reagents produced by specialized corporations but are limited by the need for purchase and the inconvenience of using and carrying them. In the present prospective study, four accessible odors common in daily life (essential balm, vinegar, alcohol, and rose perfume) were selected, and the therapeutic effects and influence factors during olfactory training on post-URTI olfactory dysfunction were investigated.

## DATA AND METHODS

### Clinical data

A total of 131 outpatients with URTI-induced olfactory dysfunction treated at the Department of Otolaryngology at Shanxi People's Hospital between December 2015 and August 2018 were enrolled in the present study. These patients were randomly divided into two groups, according to the combinations of olfactory agents: test group and control group. The present study was approved by the Ethics Committee of the hospital. All subjects included provided signed informed consent.

The inclusion criteria were as follows: (1) definite history of URTI and secondary olfactory dysfunction after infection, without a blank period between the two, and the course of olfactory dysfunction was ≤24 months; (2) detailed inquiry of medical history to exclude history of traumas, Alzheimer's disease, Parkinson's disease, mental diseases, and immune diseases; (3) nasal endoscopic examination to eliminate nasal neoplasm, nasal sinusitis, allergic rhinitis, olfactory cleft edema, and other nasal diseases; (4) sinal computed tomography (CT) and head magnetic resonance imaging (MRI) to exclude space-occupying diseases in the nasal cavity, sinus and intracalvarium, as well as neurodegenerative diseases; (5) uncured by medication of glucocorticoid, gingko extracts, or vitamin A, and time of drug therapy was >1 month.

The exclusion criteria were as follows: contraindication to therapeutic method or drugs; interruption due to intolerance or adverse reactions during therapy; development of other diseases or need of other drugs that might interfere with the therapeutic effects.

### Methods

#### Medical data collection

Information was collected from all patients included *via* questionnaire, including gender, age, body mass index (BMI), course of diseases, history of smoking/drinking, history of diabetes, history of hypertension, combination with taste dysfunction, and visual analog scale (VAS) score.

#### Olfactory function test

Sniffin' Sticks (Burghart, Germany) were used in the tests before treatment and at 1, 3, and 6 months after treatment. This test involved three parts: (1) odor threshold test, with a score ranging from 0 (even the highest concentration cannot be discriminated) to 16 (the lowest concentration can be discriminated); (2) odor discrimination test (a score of 16 mean that all odors can be discriminated); (3) odor identification (a score 16 mean that all odors can be identified). After the three parts of tests, the scores for odor threshold (T), odor discrimination (D), and odor identification (I) were added together, and the result was the threshold-discrimination-identification (TDI) score used to evaluate olfactory function.

#### Therapeutic scheme

The olfactory training involved four odors in the test group (essential balm, vinegar, alcohol, and rose perfume) and control group (phenyl ethanol-rose, menthol-mint, citronellal-lemon, and eugenol-clove) (Sigma-Aldrich, USA). During the treatment, each olfactory agent was smelled for 10 seconds/time, and the interval between two olfactory agents was 10 seconds. Each olfactory training lasted for five minutes, and the training frequency was one time before breakfast, and another time before sleep every day.[6] Olfactory function was tested at 1, 3, and 6 months after treatment.

#### Therapeutic effect assessment

The therapeutic effect was assessed by the variation in mean TDI scores after the treatment. A variation of >6 was considered as "effective".[7]

### Statistical analysis

Statistical analysis was conducted on SPSS 20. With the clinical effect as the dependent variable,

Logistic regression analyses were performed with the independent variables of gender, age, BMI, course of diseases, history of smoking/drinking, history of diabetes, history of hypertension, combination with taste dysfunction, and VAS score. The TDI scores before and after treatments were compared between groups *via* paired t-test.

## RESULTS

### Basic information

Among the 136 patients (68 patients in each group), 11 patients (eight patients from the control group and three patients from the test group) were excluded due to treatment interruption (nine patients for reasons such as being on business) or missed follow-up (two patients). Finally, 65 tested cases and 60 controls were included in the present study. In the test group, the 66 patients comprised 21 males and 44 females. Their ages ranged within 18-66 years old (50.2 ± 13.5 years old), and their course of diseases lasted within 6.0-22.0 months (11.9 ± 4.8 months). The numbers of patients with a BMI of ≥24, a history of drinking, diabetes, hypertension, and complication by taste dysfunction were 21 (32.3%), 10 (15.4%), 13 (20.0%), 18 (27.7%), and 20 (30.8%), respectively. The VAS score was 4.18 ± 1.84. In the control group, the 60 subjects comprised 20 males and 40 females. Their ages ranged within 25-65 years old (52.4 ± 12.3 years old), and their course of diseases lasted within 6.0-21.0 months (13.4 ± 4.8months). The numbers of patients with a BMI of ≥24, a history of drinking, diabetes, hypertension, and complication by taste dysfunction were 22 (36.7%), 11 (18.3%), 14 (23.3%), 17 (28.3%), and 19 (31.7%), respectively. The VAS score was 4.13 ± 1.87. In terms of age and gender, the patients included were mostly old women (67.2% females). This was consistent with another study that reported that URTI-induced olfactory dysfunction mostly affects women over 50 years old.[8] Olfactory dysfunction was dominated by hyposmia and anosmia (test group: 47 and 18 patients, respectively; control group: 43 and 17 subjects, respectively). The TDI scores were dominated by the deterioration of olfactory identification ability.[9]

### Clinical therapeutic effect

The effectiveness rates at 1, 3, and 6 months after treatment were 3.08%, 26.15%, and 41.54%, respectively, in the test group, and 1.67%, 26.67%, and 41.67%, respectively, in the control group. No significant difference in TDI scores was found between both groups at any time point ($P$>0.05). Furthermore, the TDI scores did not significantly change after one month of treatment in either of the groups ($P$>0.05). After 3 and 6 months of treatment, the TDI scores both significantly increased, and the odor discrimination and identification abilities were significantly strengthened for both groups ($P$<0.05), but the odor thresholds did not improve (Table 1 and Fig. 1).

### Influencing factors on the clinical effect

The single-factor analysis revealed that the course of the disease was significantly correlated with the therapeutic effect for both groups. Patients with a shorter course of the disease (time from the onset of symptoms to the start of olfactory training) had a significantly better therapeutic effect (test group: OR= 1.374, CI: 1.135-1.663, $P$ =0.001; control group: OR=0.805, CI: 0.696-0.931, $P$=0.004; Table 2).

## DISCUSSION

URTI is one of the common causes of olfactory dysfunction, which impacts the quality of life, social communication, and nutrient ingestion, and even causes depression or other mental problems.[10,11] The possible infection mechanisms may be correlated to the following: a reduction in the number of olfactory receptors and olfactory tracts, and the loss of olfactory receptor cilium due to viral infection; the replacement of the olfactory epithelia by epithelia, or massive scaring; olfactory pathway invasion into the olfactory center caused by a neurotropic virus. As reported, URTI-induced olfactory dysfunction is dominated by hyposmia, and mainly affects women over the age of 50 years old. This may be accompanied by taste dysfunction, but not with other nasal symptoms. The Sniffin' Sticks tests mostly revealed that the deterioration of olfactory identification ability was more significant. The present study reveals that the 125 included patients are mostly females (67.2%), aged 51.26 ± 12.94 years old. The olfaction psychophysics tests revealed that olfactory dysfunction was dominated by hyposmia (90 patients, 72.0%). The TDI scores show that the deterioration of odor identification ability was more evident. These results are consistent with previous research. During clinical practice in recent years, olfactory training has been increasingly used to treat URTI-induced olfactory dysfunction. Since olfactory training is a novel method

of olfactory dysfunction treatment, the relationship between olfactory agent selection and clinical effect should be explored and enriched by relevant medicine-based evidence. To date, the olfactory agents used in research are standardized reagents made from specialized corporations, which are stored in liquid-exclusive glass bottles, making these inconvenient for long-distance carrying. Unfortunately, many patients have to interrupt treatment, which reduces therapeutic compliance. For this reason, the investigators selected four accessible odors found in daily life (essential balm, vinegar, alcohol, and rose perfume) for the olfactory training.

In the novel treatment of olfactory training, the olfaction of the olfactory dysfunction patient is periodically irritated by olfactory agents to recover olfactory function. The review of relevant studies revealed that olfactory training may be a new effective intervention for olfactory dysfunction patients, and that its effective rate is 28%-63%.[12]

It is reported that olfactory training has been confirmed to be effective for patients with postinfectious olfactory loss. Besides, compared with the classical odor training group, the modified olfactory training group could improve the success rate of this therapy by increasing the duration of olfactory training and

**TABLE 1.** THE TDI SCORES BEFORE AND AFTER TREATMENT OF CONTROL GROUP AND TEST GROUP

| Time | Control group | | | | Test group | | | |
|---|---|---|---|---|---|---|---|---|
| | T | D | I | TDI | T | D | I | TDI |
| Before treatment a | 6.76±1.96 | 7.17±1.74 | 2.88±1.51 | 16.82±2.67 | 6.49±2.18 | 7.06±1.85 | 2.82±1.60 | 16.29±2.69 |
| 1 month after treatment b | 6.88±2.15 | 7.32±1.85 | 2.75±1.67 | 17.30±2.96 | 6.57±2.05 | 7.20±1.72 | 2.88±1.56 | 16.65±2.55 |
| 3 months after treatment c | 6.91±2.03 | 8.70±1.96 | 4.92±1.71 | 20.53±3.01 | 6.58±2.07 | 8.63±1.92 | 5.00±1.71 | 20.43±2.94 |
| 6 months after treatment d | 6.86±2.35 | 9.48±2.18 | 6.13±1.62 | 22.48±3.73 | 6.64±2.08 | 9.66±2.36 | 6.40±1.75 | 22.88±3.90 |
| Ta-b (P) | -1.121 | -1.454 | 0.893 | -1.819 | -0.751 | -1.218 | -0.540 | -1.695 |
| | (P>0.05) | P>0.05 | P>0.05 | P>0.05 | (P>0.05) | P>0.05 | P>0.05 | P>0.05 |
| Ta-c (P) | -1.501 | -12.091 | -11.047 | -11.065 | -0.642 | -12.103 | -14.106 | -13.067 |
| | (P>0.05) | (P<0.05) | (P<0.05) | (P<0.05) | (P>0.05) | (P<0.05) | (P<0.05) | (P<0.05) |
| Ta-d (P) | -0.799 | -16.134 | -17.561 | -15.400 | -1.045 | -12.953 | -18.290 | -15.233 |
| | (P>0.05) | (P<0.05) | (P<0.05) | (P<0.05) | (P>0.05) | (P<0.05) | (P<0.05) | (P<0.05) |
| Tb-c (P) | -0.333 | -10.609 | -13.716 | -10.893 | -0.133 | -11.920 | -13.861 | -13.450 |
| | (P>0.05) | (P<0.05) | (P<0.05) | (P<0.05) | (P>0.05) | (P<0.05) | (P<0.05) | (P<0.05) |
| Tb-d (P) | 0.063 | -14.755 | -20.112 | -13.257 | -0.516 | -12.956 | -18.607 | -15.170 |
| | (P>0.05) | (P<0.05) | (P<0.05) | (P<0.05) | (P>0.05) | (P<0.05) | (P<0.05) | (P<0.05) |
| Tc-d (P) | 0.375 | -7.176 | -8.045 | -6.765 | -0.424 | -7.090 | -8.787 | -7.918 |
| | (P>0.05) | (P<0.05) | (P<0.05) | (P<0.05) | (P>0.05) | (P<0.05) | (P<0.05) | (P<0.05) |

a= before treatment, b= 1 month after treatment, c= 3 months after treatment, d= 6 months after treatment

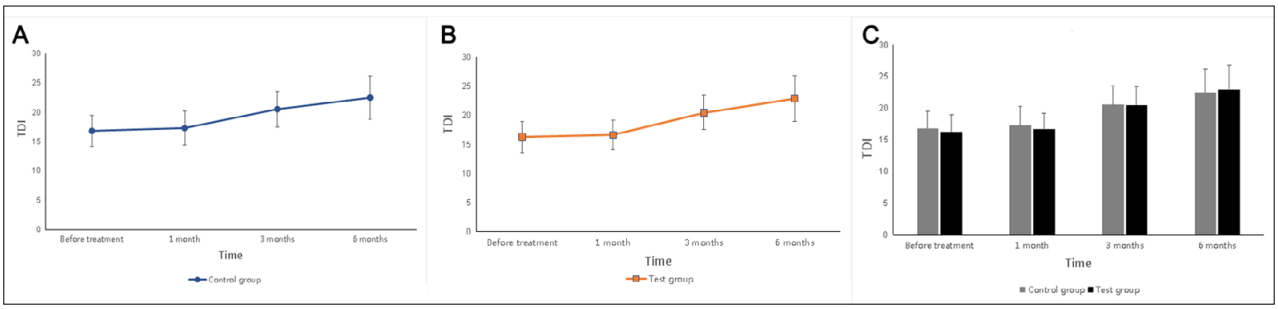


**FIGURE 1.** TDI SCORES DATA AND COMPARISOIN

A: TDI scores data in control group; B: TDI scores data in test group; C: Comparison of TDI scores between control and test group.

changing the odors.[13] The olfactory system of mammals can be regenerated during their whole life, and olfactory epithelia and olfactory bulbs both have strong regeneration ability.[14] A higher olfactory center has moderate regeneration ability, which theoretically underlies the treatment of olfactory dysfunction by olfactory training. Recent research showed that recurrent olfactory irritation can intensify the potential reaction of olfactory epithelia, indicating that olfactory training is involved in olfactory epithelial reconstruction, probably by increasing the number of olfactory neurons in humans.[15] In addition to the above direct participation, olfactory training can also significantly enlarge the volumes of olfactory bulbs[16] and improve the network connection of the olfaction-related cerebral cortex[17,18], indicating that olfactory training is critical in regenerating the central nervous system.

The present subjects were URTI-induced olfactory dysfunction patients who had not been cured by drugs. Since unpleasant odors *vs.* pleasant odors can more significantly affect the breathing mode of humans[19] and reduce the work memory ability of a part of normal people[20], relatively pleasant smells were selected for the present olfactory training. The effective rates at 1, 3, and 6 months after treatment were 3.08%, 26.15%, and 41.54%, respectively, in the test group, and 1.67%, 26.67%, and 41.67%, respectively, in the control group. The therapeutic effects were not significantly different between groups at any time point ($P>0.05$), suggesting that the two combinations of olfactory agents achieved the same therapeutic effect. The TDI scores in the 1$^{st}$ month were not significantly different from those before treatment ($P>0.05$). The scores in the 3$^{rd}$ and 6$^{th}$ months were both significantly higher than those before treatment ($P<0.05$). Pertaining to the treatment period and therapeutic effect, this result can be better explained when a relatively long period of olfactory system regeneration is considered. The potential influencing factors on the clinical effect were studied *via* Logistic regression analysis, which revealed that the major influence factor on prognosis was the course of the disease, which is consistent with previous research.[21] The odor discrimination ability and odor identification ability were both significantly improved after 3 and 6 months of training ($P<0.05$), but the odor threshold did not obviously improve ($P>0.05$). In other words, the increment of TDI scores in URTI-induced olfactory dysfunction patients after the olfactory training was mainly reflected in the changes in odor discrimination and identification abilities, but not in the odor threshold. To date, most studies have held that the olfactory threshold is mediated at the olfactory epithelium level. However, functional MRI research has confirmed that olfactory training may lead to the most apparent change in the cortex.[22] Since patients with complete anosmia are nonresponsive to olfactory irritation, it is impossible to use odor excitement to activate the olfactory functions of olfactory epithelia and the brain. The olfactory system is closely correlated to the nasal trigeminal nerve system, and most odors not only irritate the smell neurons but also activate the trigeminal nervous system.[23,24] Moreover, the nasal trigeminal nervous system is largely involved in olfactory signal processing, such as odor laterality identification and odor intensity assisted identification.[23,25,26] Thus, olfactory training can improve the odor discrimination and identification abilities of olfactory dysfunction patients, which may be correlated to the deep

**TABLE 2.** REGRESSION ANALYSIS OF INFLUENCE FACTORS CORRELATED WITH THE CLINICAL EFFECT

| Factor | Control group | | | Test group | | |
|---|---|---|---|---|---|---|
| | OR | 95%CI | P | OR | 95%CI | P |
| Gender | 0.572 | 0.142~2.306 | 0.432 | 1.842 | 0.425~7.985 | 0.414 |
| Age | 0.979 | 0.927~1.033 | 0.435 | 1.009 | 0.958~1.062 | 0.745 |
| BMI | 1.954 | 0.545~7.010 | 0.304 | 0.284 | 0.068~1.188 | 0.284 |
| Course of disease | 0.805 | 0.696~0.931 | 0.004 | 1.374 | 1.135~1.663 | 0.001 |
| History of smoking/drinking | 2.558 | 0.448~14.601 | 0.290 | 0.305 | 0.055~1.711 | 0.305 |
| Complicated with taste dysfunction | 1.795 | 0.462~6.979 | 0.399 | 0.864 | 0.212~3.515 | 0.838 |
| VAS score | 0.988 | 0.702~1.389 | 0.943 | 1.066 | 0.713~1.593 | 0.755 |
| Diabetes | 2.821 | 0.601~13.237 | 0.188 | 1.386 | 0.254~7.556 | 0.706 |
| Hypertension | 1.492 | 0.356~6.258 | 0.584 | 0.461 | 0.108~1.964 | 0.295 |
| Preoperative TDI | 1.061 | 0.825~1.364 | 0.644 | 1.056 | 0.82~1.354 | 0.671 |

participation of the nasal trigeminal nervous system. Based on these present results, it has been speculated that different types of olfactory agents may function similarly in irritating the olfactory system and nasal trigeminal system. The involvement of the trigeminal nervous system is one of the possible mechanisms for stimulating the olfactory system. There may be other mechanisms to stimulate the olfactory system. However, this needs to be confirmed through more relevant research.

Overall, these two combinations of olfactory agents are effective interventions for URTI-induced olfactory dysfunction that can significantly improve the odor discrimination and identification abilities. The inclusion rate of the test group was significantly higher than that of the control group, mainly due to the inconvenience of carrying the agents and economic reasons. When odors that can be easily obtained in daily life (e.g., essential balm, vinegar, alcohol, and rose perfume) were used in olfactory training, a similar clinical effect to the control group was achieved. Furthermore, since low cost and portability would increase patient compliance, the clinical use of these odors is recommended. Moreover, the prolonged and earlier start of olfactory training would be more helpful for the recovery of olfactory functions.


## RESUMO

OBJETIVO: *Comparar duas combinações de agentes olfativos para uso em terapia de treinamento olfativo no tratamento de disfunção olfatória após infecção do trato respiratório superior (ITRS) e investigar os fatores que influenciam os efeitos clínicos.*

METODOLOGIA: *125 pacientes com disfunção olfativa foram divididos aleatoriamente em dois grupos: teste e controle. Durante o treinamento olfativo, quatro odores foram utilizados em ambos os grupos. O treinamento olfativo durou 24 semanas. Em seguida, os participantes foram testados usando Sniffin' Sticks e o escore de discriminação, limiar e identificação (TDI) antes do tratamento e 1, 3 e 6 meses após o ele. Os escores de TDI foram comparados em momentos diferentes, entre os grupos e dentro deles, e os fatores de influência foram analisados.*

RESULTADOS: *Não houve diferença significativa nos escores de TDI entre os dois grupos. Além disso, os escores de TDI não demonstração nenhuma alteração significa após um mês de tratamento em ambos os grupos. Após 3 e 6 meses de tratamento, ambos os escores de TDI aumentaram significativamente, e as habilidades de identificação e discriminação de odores melhoraram significativamente em ambos os grupos; contudo, os limiares de odor não demonstraram melhora. O curso da doença foi um importante fator de influência no efeito terapêutico do treinamento olfativo em ambos os grupos.*

CONCLUSÃO: *A combinação de bálsamo essencial, vinagre, álcool, e perfume de rosas no treinamento olfativo, todos aromas comumente encontrados na vida cotidiana, podem efetivamente curar disfunção olfativa induzida por ITRS e melhorar significativamente as habilidades de discriminação e identificação de odores. Além disso, a prolongamento do tempo de tratamento pode ajudar na recuperação das funções olfativas, e o início antecipado do treinamento olfativo pode melhorar o efeito terapêutico.*


PALAVRAS CHAVE: *Transtornos do Olfato. Percepção Olfatória. Capacitação. Olfato/fisiologia. Infecção.*

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ORIGINAL ARTICLE

# 30 Years of Experience with Non-Hodgkin Lymphoma in Children and Adolescents: a retrospective cohort study

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## SUMMARY

OBJECTIVE: *Describe the clinical and demographic characteristics of pediatric patients with non-Hodgkin's lymphoma (NHL) enrolled in a tertiary unit of Pediatric Hematology between 1982-2015.*

PATIENTS AND METHODS: *A retrospective cohort study of 140 patients aged 16 years or less with NHL. Demographic characteristics, data on diagnosis, and outcomes were analyzed. The overall survival (OS) analysis and stratification by the most frequent histological subtypes were performed using the Kaplan-Meier method.*

RESULTS: *One hundred and thirty-six patients with de novo NHL and four with NHL as a second malignancy were analyzed. The median age at diagnosis was 6.4 years (interquartile range, 4.2 to 11.1 years); 101 patients were males. Four patients had primary immunodeficiency, four had human immunodeficiency virus, two post-liver transplantation, and one had autoimmune lymphoproliferative syndrome. The most frequent histological type was NHL of mature B- cell (B-NHL-B; 67.1%), with Burkitt's lymphoma being the most frequent subtype, and lymphoblastic lymphoma (LBL, 21.4%). The main clinical manifestation at the diagnosis was abdominal tumors (41.4%). During the follow-up time, 13 patients relapsed, but five of them reached a second remission. Thirty-five patients died, and 103 remained alive in clinical remission. No contact was possible for two patients. The OS at 5 years was 74.5% (± 3.8%). The OS estimated for patients with LBL, NHL-B, and the remaining was 80.4%±7.9%, 72.8%±4.7%, and 74.5%±11%, respectively (P = 0.58).*

CONCLUSION: *Our results are comparable with cohorts from other middle-income countries.*

KEYWORDS: *Lymphoma, Non-Hodgkin. Child. Cohort Studies.*

## INTRODUCTION

In Brasil, a middle-income country (MIC), cancer is already the leading cause of death (8% of total) due to illness among children and adolescents aged 1 to 19 years. According to the Brasilian Institute of Cancer (INCA), it is expected that 12.5 thousand new cases of cancer in the pediatric population will be diagnosed

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over the next years (http://www2.inca.gov.br). Lymphomas are the second most common cancer in Brazilian children, and non-Hodgkin lymphoma (NHL) is the most frequent histological type[1].

For more than 30 years, the Division of Pediatric Hematology of the Hospital of Clinics of University Federal of Minas Gerais (HC-UFMG) has provided care to children and adolescents with NHL[2,3]. The HC-UFMG is located in Belo Horizonte, capital of the state of Minas Gerais, the third most populous municipality in the Southeastern region of Brasil [4]. As a tertiary care center, systematic data obtained during the clinical course of these patients is a rich source of knowledge to understand the outcome of NHL. The aim of this retrospective cohort study was to describe the main clinical characteristics and outcomes of 140 patients with NHL followed up at a single tertiary center in a MIC.

## METHODS

Data were collected retrospectively from medical records of the Division of Pediatric Hematology Unit – HC-UFMG of 140 patients aged up to 16 years with NHL admitted between 1981 and 2015. Medical records were reviewed to collect demographic data (age, gender), clinical data (medical history, physical examination, clinical presentation), diagnostic procedures (imaging studies, bone marrow aspiration, cerebrospinal fluid - CSF analysis, tumor biopsy), disease staging, laboratory data (lactate dehydrogenase - LDH levels, blood counts, serum electrolytes, liver and kidney tests), treatment, and outcomes. All the diagnoses were confirmed by morphological and immunohistochemistry criteria defined by the World Health Organization (WHO) classification[5]. Immunohistochemistry was performed using monoclonal antibodies CD20, CD10, CD79a, CD30, CD3, CD15, TdT, CD45, and CD45RO for the detection of B and T cells. Cell lineage assignment required 50% or more of positive neoplastic B or T cells. Clinical staging was based on the St. Jude Children's Research Hospital staging system [6]. Central nervous system (CNS) disease was diagnosed by the presence of morphologically identifiable lymphoma cells (regardless of quantity) in CSF, an intracranial mass, or cranial nerve palsy not caused by an extradural mass.

Patients were treated according to their histological subtype: for lymphoblastic lymphoma (LBL), protocols based on ALL-type (acute lymphoblastic leukemia) strategy, for B-NHL (lymphoma non-Hodgkin of B- cell) and ALCL (anaplastic large cell lymphoma), short pulse-intensive chemotherapy[7].

Complete remission (CR) was defined as the disappearance of all tumor masses confirmed by clinical examination and imaging investigations, approximately one month after the end of the treatment. Progression of the local tumor was defined if the tumor site showed no decrease in size after starting chemotherapy. Relapse was defined as the recurrence of lymphoma with the same histological or immunophenotype features as the initial one at any site after CR was achieved. Local relapse was diagnosed when it involved a previously involved site (except bone marrow and CSF).

The data were stored in a database developed for this specific purpose. Data analysis was descriptive and performed in the SPSS program. Data are reported as medians and interquartile range (IQ) or means and standard deviation (SD), when appropriate. Overall survival (OS) was defined as the time from diagnosis to the date of death due to any cause, or the date of last follow-up contact for patients who were alive. The OS was analyzed using the Kaplan–Meier method. OS analysis was performed stratified by the most frequent histological subtypes.

The study was approved by the Research Ethics Committee of UFMG.

## RESULTS

### Patient characteristics

The median age at diagnosis was 6.4 years (IQ range, 4.2 to 11.1). The diagnosis was based on the cytological examination of abdominal effusion in two patients, pleural effusion in five patients, and on tumor biopsies for the other cases. According to immunohistochemistry or immunophenotyping, 108 patients (77.1%) had B-cell lineage neoplasia, 25 patients had T-cell, and 7 were indeterminate. Four patients had primary immunodeficiency (one with ataxia-telangiectasia), four acquired immunodeficiency syndrome (AIDS), two post-transplantation NHL, and one had autoimmune lymphoproliferative syndrome (ALPS). NHL arising in skin, bone, and liver were diagnosed in seven, four, and one patient, respectively. One patient diagnosed with cutaneous NHL had AIDS.

The most common histologic subtypes of NHL were mature B-cell (B-NHL) in 94 patients (67.1%), including Burkitt' lymphoma (BL - 69 patients, 49.2%),

mature B-cell leukemia (B-AL, 6 patients), diffuse large B-cell lymphoma (DLBCL, 11patients), T-cell rich B-cell lymphoma (TCRBCL, 5 patients) and three undetermined. Thirty patients (21%) were diagnosed with lymphoblastic lymphoma (LBL) and four with anaplastic large cell lymphoma (ALCL- 2- kinase - ALK-positive). The characteristics of these patients are summarized in Table 1.

Abdominal tumor, occurring in 58 patients (41.4%), was the most common clinical presentation, followed by peripheral lymphadenopathy (32 patients, 22.9%), mediastinal involvement (17 patients, 12.1%), and face (7 patients, 5%). Other involved organs included the central nervous system (2), paravertebral mass (2), bone (4), skin (7), liver (1), and others (3). Five patients had more than one site of involvement: four had the involvement of the abdomen and thorax, and one had the involvement of the abdomen and paravertebral region. In two patients, no information on initial clinical manifestation had been recorded in clinical files. Most patients had advanced disease (88 patients, 62.9%).

**TABLE 1.** CLINICAL AND DEMOGRAPHIC CHARACTERISTICS OF 140 PATIENTS WITH NHL

| | N (%) |
|---|---|
| Age of diagnosis (yr) | |
| Median (IQ25-75) | 6.4 (4.2 – 11.1) |
| Gender | |
| Male | 101 (72.1) |
| Female | 39 (27.9) |
| Clinical presentation | |
| Abdominal tumors | 58 (41.4) |
| Peripheral lymphadenopathy | 32 (22.9) |
| Mediastinal | 17 (12.1) |
| Face | 7 (5.0) |
| More than one site of involvement | 5 (4.2) |
| Skin | 7 (5.0) |
| Bone | 4 (2.9) |
| Others | 8 (5.7) |
| No information | 2 (1.4) |
| Cell origin | |
| B cell | 108 (77.1) |
| T cell | 25 (17.9) |
| Indeterminate | 7 (5.0) |
| Histological type | |
| B-NHL | 94 (67.1) |
| Burkitt Lymphoma | 69 (49.2) |
| Mature B-cell Acute Leukemia | 6 (4.3) |
| Diffuse Large B-Cell Lymphoma | 11 (7.9) |
| T-Cell rich B-Cell Lymphoma | 5 (3.6) |
| Indeterminate | 3 (2.1) |
| Lymphoblastic Lymphoma | 30 (21.4) |
| B-LBL | 7 (5.0) |
| T-LBL | 18 (12.8) |
| Indeterminate | 5 (3.6) |
| Anaplastic Large Cell Lymphoma | 4 (2.9) |
| Cutaneous lymphoma* | 7 (5.0) |
| Osseous lymphoma† | 4 (2.9) |
| Hepatic lymphoma (indeterminate) | 1 (0.7) |
| Stage | |
| Localized | 35 (25.0) |
| Advanced | 88 (62.9) |
| No information | 17 (12.1) |
| Outcome | |
| Alive in remission | 103 (73.6) |
| In first remission | 98 (70.0) |
| After relapsing¶ | 5 (3.6) |
| Death | 35 (25.0) |
| Primary event | 27 (19.3) |
| After relapsing¶ | 8 (5.7) |
| No information | 2 (1.4) |

* T-cell: 6; indeterminate: 1; † B-cell: 3; indeterminate: 1; ¶ Relapse rate: 13/140 (9.3%).

### Outcome

During the follow-up, 13 patients (9%) relapsed, 35 died (25%), and 103 (72%) remained alive in disease-free clinical remission. Two patients were not found for the follow-up (Table 1). The estimated OS for all patients was 74.5% ± 3.8% (Figure 1). The likelihood of OS for patients with LBL, B-NHL, and other subtypes was 80.4%±7.9%, 72.8%±4.7%, and 74.5%±11%, respectively (P=0.58; Figure 2).

## DISCUSSION

NHL is a heterogeneous group of highly malignant tumors with a distinct pathological immune framework and clinical features[8]. In this retrospective cohort study, we evaluated the clinical outcome of 140 pediatric patients with NHL enrolled in a single tertiary center in a Southeastern region in Brasil.

The overall incidence of pediatric NHL is not accurately known. It comprises approximately 8-10% of all malignancies in children aged between five and 19 years, and it is more frequent in male adolescents [8-10]. The annual incidence per million inhabitants ranges from 5.9 in children younger than 5 years of age to about 10 in children between 5 and 14 years old, and 15 in adolescents [10]. It has been estimated that 90% of children diagnosed with NHL live in low-middle income countries (LMIC)[11]. In Brasil, NHL is also more prevalent among male pediatric patients[1]. In our study, NHL was about 2.5 times more frequent in boys.

The etiology of NHL is not well known. Like cancer in general, NHL probably arises from interactions between exogenous or endogenous exposures, genetic susceptibility, and chance [12]. On the other hand, it is well known that immunodeficiency, either primary or secondary, increases the risk of NHL [10]. Recently described is the association of interleukin IL-10 receptor deficiency with childhood B-NHL and the increased risk of NHL in the constitutional mismatch

**FIGURE 1.** KAPLAN MEIER ESTIMATES THE PROBABILITY OF OVERALL SURVIVAL AMONG 140 CHILDREN AND ADOLESCENTS WITH NHL.

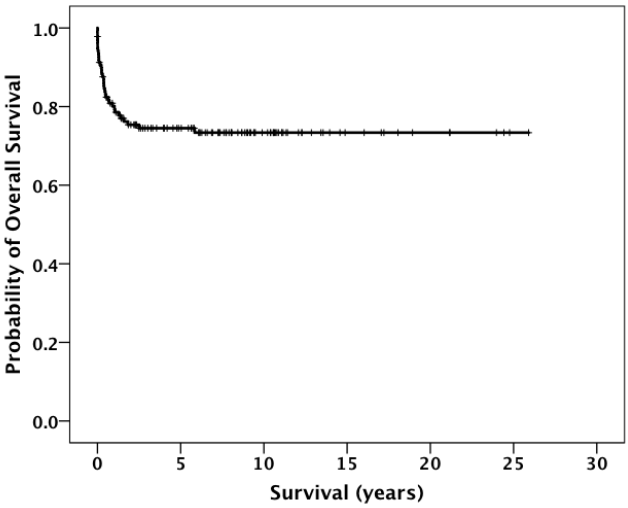
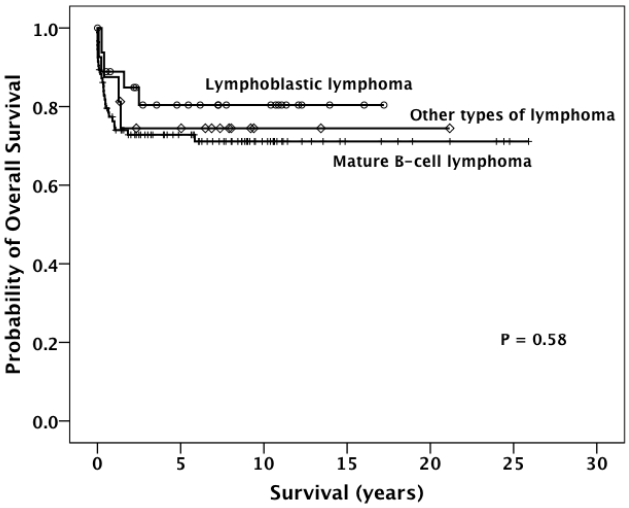



**FIGURE 2.** KAPLAN MEIER ESTIMATES THE PROBABILITY OF OVERALL SURVIVAL STRATIFIED ACCORDING TO THE HISTOLOGICAL SUBTYPES.




repair deficiency syndrome[13]. In the present cohort, the majority of patients were immunocompetent, and only 11 (8%) had immunosuppression or immunodeficiency-related NHL.

There have been scarce studies concerning the association between race/ethnicity and the incidence of pediatric NHL. The Pediatric Health Information System (USA) reported that NHL is almost twice as common in whites compared to African Americans [14]. Brazilians constitute a trihybrid population with European, African, and Amerindian roots. In the population of the state of Minas Gerais, where the present study was conducted, European-African interbreeding occurred very intensely [15]. In Brasil, differences in NHL incidence by race/ethnicity are not reported.

The major histologic subtype of NHL in high-income countries (HIC) is Burkitt lymphoma (BL, 40%), followed by lymphoblastic lymphoma (LBL, 25%), diffuse large B-cell lymphoma (LDGCB- 10%), and anaplastic large cell lymphoma (ALCL,10%)[8,11]. In LMIC as Egypt, Nicaragua, Brasil, and tropical Africa, BL is also the most frequent subtype of NHL [1,11]. In contrast, in India, T-LBL is the most common NHL subtype (32%)[16]. In the present study, 67% of cases of NHL had B-cell origin, and BL was the most common subtype of NHL, as emphasized in the literature. The predominance of BL (49.2%) was also reported by another Brasilian study conducted in the Northeastern region of Brasil [17]. In the present cohort, the median age at diagnose was lower than in HIC[9]. The median age at presentation of NHL in children in HIC is 10 years, while in our cohort, it was about six years.

As reported in a study of cancer survivors diagnosed in childhood and adolescence, NHL as a secondary neoplasm (SN) is rare and information on characteristics and outcomes of these patients are scarce [18]. In the present study, only four patients were diagnosed with NHL following previous cancers other than NHL. In all patients, the first cancer was a lymphoid neoplasm.

The clinical features of NHL in children are characterized mostly by extranodal involvement and are related to the histological type. The abdominal tumor was the most frequent feature in our patients, reflecting the very high incidence of BL. This clinical manifestation is also the most frequent in the USA among children with BL. In Africa, where BL is endemic, tumors in the jaw or around the eyes are more common[19]. Conversely, NHL arising in sites like bone, skin, and liver is unusual and has been reported in approximately 7% of all NHL pediatric patients [20,21]. In the present series, only 12 patients (8.6%) exhibited an involvement of these less common sites at the time of the initial diagnosis. Regarding CNS involvement, the exact incidence is unknown. In a study by Salzburg et al., [22] 6% of patients with NHL were CNS positive, and the most frequent subtypes of NHL were BL/ B-AL. In our study, only 1.4% of patients were CNS positive, and both had BL.

NHL is considered one of the neoplasms with the best chance of cure in children, although it is almost exclusively a high-grade malignancy with disseminated disease at diagnosis [9]. Currently, depending on the histological type, most patients in HIC can be cured by risk-adapted chemotherapy and supportive

care[23]. This modern chemotherapy requires supportive care to face life-threatening toxicities, sometimes unavailable in several LMIC. While the estimated 5-year survival rate ranges from approximately 70% to >95%, depending on stage and histology in HIC, in LMIC, the overall survival is less than 50% [9,11]. This discrepancy between LMIC and HIC has been attributed to several problems such as later diagnosis, misdiagnosis, pathology capacity, poor clinical status of the patient at diagnosis, the non-adherence to treatment, unavailability of some active drugs, and inadequate supportive care. In the present cohort, the probability of 5-year OS for 140 patients with NHL was 74.5% (±3.8%), which is comparable to those reported in other middle-income countries. Regarding the most frequent histological types in the present cohort, we did not detect a statistically significant difference of OS between those with LBL and B- NHL when compared with the other patients. Interestingly, by inspecting the Kaplan-Meier plots concerning OS, we can observe that there was a sharp drop within the first two years after the diagnosis. Of note, patients who survived this period did not present any events thereafter. This possibly means that a delayed referral may play a substantial role in the worse prognosis in our cohort once patients could be admitted in worse clinical conditions.

For patients who experience relapse, the prognosis is clearly worse and differs according to the NHL subtype. Patients with relapsed ALCL or DLBCL have a fair chance to survive (40-60%), whereas the survival of patients with relapsed BL and LBL is less than 30%[24]. In the present cohort, 13 patients relapsed, and eight of them (61.5%) died.

Although the present study has a significant number of patients, it has some limitations that deserve mention. The major limitation are issues inherent to all retrospective cohort studies, in which some clinically important details may be unavailable. The limited number of some histologic types of NHL (DLBCL, ALCL) and NHL arising in unusual sites in our cohort lead to insufficient power to draw definitive conclusions. On the other hand, the diagnoses of rare subtypes such as TCRBCL can be attributed to better training with diagnostic procedures. Despite these limitations, our study was carried out by the same medical team in a single institution with long follow-up time. The adherence to treatment of patients and their families was impressive for a MIC region.

## CONCLUSION

In conclusion, our results can contribute to the understanding of the epidemiology and clinical course of pediatric NHL in Brasil. The clinical features and survival rates in the cohort are comparable with those from other middle-income countries. Our findings suggest that a timely referral might contribute to a better prognosis for these patients. Further collaborative prospective studies are obviously necessary to establish the best interventions for children with NHL in middle-income countries and to develop strategies to facilitate the access of patients to the health service, reducing the early mortality related to NHL.

### Disclosure

The authors declare no conflicts of interest.

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### Authors' Contributions

Conception and study design: MCLO, MBV; data acquisition: KCS,ACB,MKC,MM,RG, AALF; data analysis/interpretation: MCLO, KCS,ACB,MKC,MM,MBV; statistical analysis: MCLO, supervision or mentorship: MCLO, MBV. Each author contributed with important intellectual content during the manuscript drafting or revision and accepted accountability for the overall work by ensuring that questions pertaining to the accuracy or integrity of any portion of the work are appropriately investigated and resolved. MCLO takes responsibility that this study has been reported honestly, accurately, and transparently and that no important aspects of the study have been omitted.

## RESUMO

OBJETIVO: *Descrever as características clínicas e demográficas de pacientes pediátricos com linfoma não Hodgkin (LNH) em uma unidade terciária de Hematologia Pediátrica entre 1982-2015.*

PACIENTES E MÉTODOS: *Estudo de coorte retrospectivo de dados de prontuários de 140 pacientes com idade até 16 anos com LNH. Características demográficas e dados relativos ao diagnóstico e evolução foram analisados. A sobrevida global (SG) e estratificada pelos subtipos histológicos mais frequentes foi analisada pelo método de Kaplan-Meier.*

RESULTADOS: *Dados de 136 pacientes com LNH de novo e quatro com LNH como segunda neoplasia foram analisados. A mediana de idade ao diagnóstico foi 6,4 anos (intervalo interquartil: 4,2 a 11,1 anos); 101 pacientes eram meninos. Onze pacientes apresentavam imunodeficiência (quatro primária, quatro secundária ao vírus da imunodeficiência humana adquirida, dois pós-transplante hepático e um com síndrome linfoproliferativa autoimune). Os tipos histológicos mais frequentes foram o LNH de células B madura (LNH-B, 67,1% dos pacientes), sendo o linfoma de Burkitt o subtipo mais frequente, e o linfoma linfoblástico (LL, 21,4%). A principal manifestação clínica ao diagnóstico foi massa abdominal (41,4%). A mediana de seguimento dos sobreviventes foi 7,7 anos (intervalo interquartil: 3,3 a 10,9 anos). Treze pacientes recidivaram (cinco alcançaram segunda remissão clínica), 35 faleceram, 103 permanecem vivos em remissão completa e dois perderam o seguimento. A probabilidade de SG em cinco anos foi 74,5%±3,8%. Para os pacientes com LL, LNH-B e os demais, a SG foi 80,4%±7,9%, 72,8%±4,7% e 74,5%±11%, respectivamente (P=0,58).*

CONCLUSÃO: *Nossos resultados são comparáveis aos de outros países de renda média.*


PALAVRAS-CHAVE: *Linfoma não Hodgkin. Criança. Estudos de coortes.*

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ORIGINAL ARTICLE

# The use of high-resolution MRI to detect thrombosis and lipid-rich carotid artery plaques in a patient with homozygous familial hypercholesterolemia

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**SUMMARY**

*Homozygous familial hypercholesterolemia is a rarely agentic disorder of the lipoprotein metabolism intimately related to premature atherosclerotic cardiovascular disease that can lead to high disability and mortality. Homozygous familial hypercholesterolemia typically affects not only the aortic root, compromising the coronary ostia, but also affects other territories such as the carotid, descending aorta, and renal arteries. Multi-contrast high-resolution magnetic resonance imaging (MRI) provides a validated and useful method to characterize carotid artery atherosclerotic plaques quantitatively. However, very few studies have been done on assessing plaque composition in patients with Homozygous familial hypercholesterolemia using high-resolution MRI. This report is to evaluate the value of MRI in accessing carotid artery disease in patients with Homozygous familial hypercholesterolemia. We describe a 28-year-old patient from Beijing, China, who presented to the Neurology Clinic with intermittent blurred vision of the right eye, headache, nausea, and vomiting for eight years without obvious causes. Familial hypercholesterolemia was suspected based on medical history and laboratory examination. Carotid Doppler ultrasound showed bilateral common carotid artery, internal carotid artery, and external carotid artery wall thickening with hyperechoic signals. Subsequently, high-resolution multi-contrast MRI of the carotid showed calcification with hypo-intense areas located at the middle layer of the plaque, with moderate stenosis. The plaque located at the right bifurcation of the common carotid artery extended to the internal carotid artery, causing lumen stenosis close to occlusion. The patient was treated with right carotid artery endarterectomy. At a 6-month follow-up, there had been no recurrence of the patient's symptoms.*

KEYWORDS: *Magnetic Resonance Spectroscopy. Plaque, Atherosclerotic. Carotid Intima-Media Thickness. Carotid Arteries. Hyperlipoproteinemia Type II.*

## INTRODUCTION

Homozygous familial hypercholesterolemia (HoFH) characterized clinically by plasma cholesterol levels >13 mmol/L, extensive xanthomas, and followed by markedly premature and progressive atherosclerotic cardiovascular disease (ACVD) is a rare and life-threatening disease[1]. ACVD typically affects the aortic root, compromising the coronary ostia, but also other territories such as the carotid, descending aorta, and

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renal arteries[2]. Cholesterol and calcium deposits, as well as artery wall fibrosis and inflammation, can lead to lumen stenosis[3]. In the last two years, several guidelines have been published in an attempt to improve FH diagnosis and treatment[4,5]. Multi-contrast high-resolution magnetic resonance imaging (MRI) provides a validated and useful method to characterize carotid artery atherosclerotic plaques[6]. However, little is known about the usefulness of multi-contrast high-resolution MRI in HoFH patients[7]. We describe the use of high-resolution MRI and plaque histology in a patient with HoFH.

## CASE PRESENTATION

The patient was a 28-year-old woman from Beijing, China, who presented to the Neurology Clinic with intermittent blurred vision of the right eye, headache, nausea, and vomiting for the past 8 years without obvious causes. The medical history revealed that the patient already had skin and joint xanthomas at the age of one year. HoFH was suspected at the age of 3, when blood examination revealed plasma cholesterol >20 mmol/L, and hyperlipidemia was found in both parents. The patient's family could not afford genetic analysis or LDL-apheresis. After several years of conventional statin treatment, plasma cholesterol levels remained high, between 13 and 15 mmol/L. The patient underwent percutaneous coronary intervention (PCI) at the age of 12, and coronary CT angiography showed diffuse lesions in the coronary artery with more than 50% stenosis (Fig. 1 A-D) as well as calcified lesions at the aortic root and coronary artery orifice (Fig. 1 E). We suspected that the patient's current symptoms of intermittent blurred vision might be caused by carotid artery atherosclerosis.

Therefore, carotid color Doppler ultrasound, intracranial Doppler ultrasound, and carotid artery CT angiography were performed. Carotid Doppler ultrasound showed bilateral common carotid artery (CCA), internal carotid artery (ICA), and external carotid artery (ECA) wall thickening with hyperechoic signals. The bifurcation of the right CCA showed hyperechoic plaque, with a maximum plaque thickness of 4.9 mm; the lesion extended to the right ICA, with maximum stenosis of about 90%. Right ECA stenosis was 75%, while that of the left CCA, ICA, and ECA was 70%. Transcranial Doppler ultrasound showed near occlusion of the right intracranial ICA. The right ophthalmic artery had a reverse flow, with the right ECA through the ophthalmic artery reversing ICA supply, as well as posterior communicating artery opening. Carotid artery CT angiography revealed the same degree of stenosis as the ultrasound, with plate-like calcification located at the bifurcation of the right CCA, proximal ICA, and ECA (Fig. 2 A-C).

Subsequently, high-resolution multi-contrast MRI was used to further evaluate carotid plaque components and vulnerability. Imaging was performed on a 3T MRI scanner using specially-designed phased-array

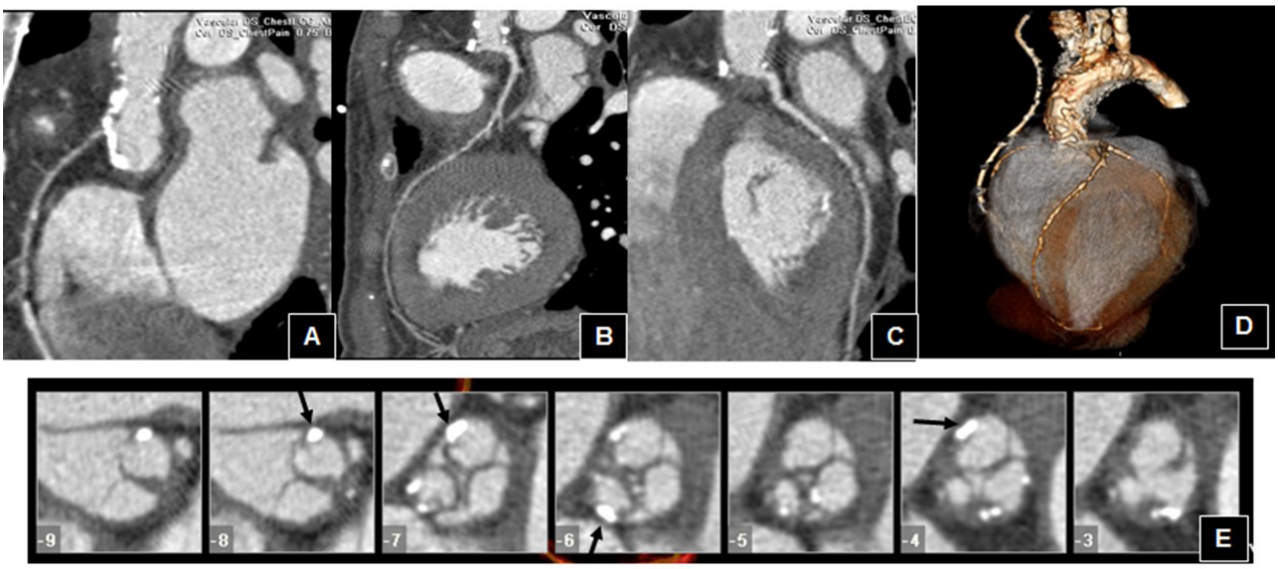


**FIGURE 1.** COMPUTED TOMOGRAPHY ANGIOGRAPHY OF THE PATIENT.
A-C, CALCIFIES, NON-CALCIFIED, AND MIXED PLAQUES IN THE MIDDLE AND DISTAL RCA, LAD, AND LCX; D, BYPASS FROM IMA TO RCA; E, IMAGES SHOWING CALCIFIED PLAQUES ON THE AORTIC VALVE (RED ARROWS)

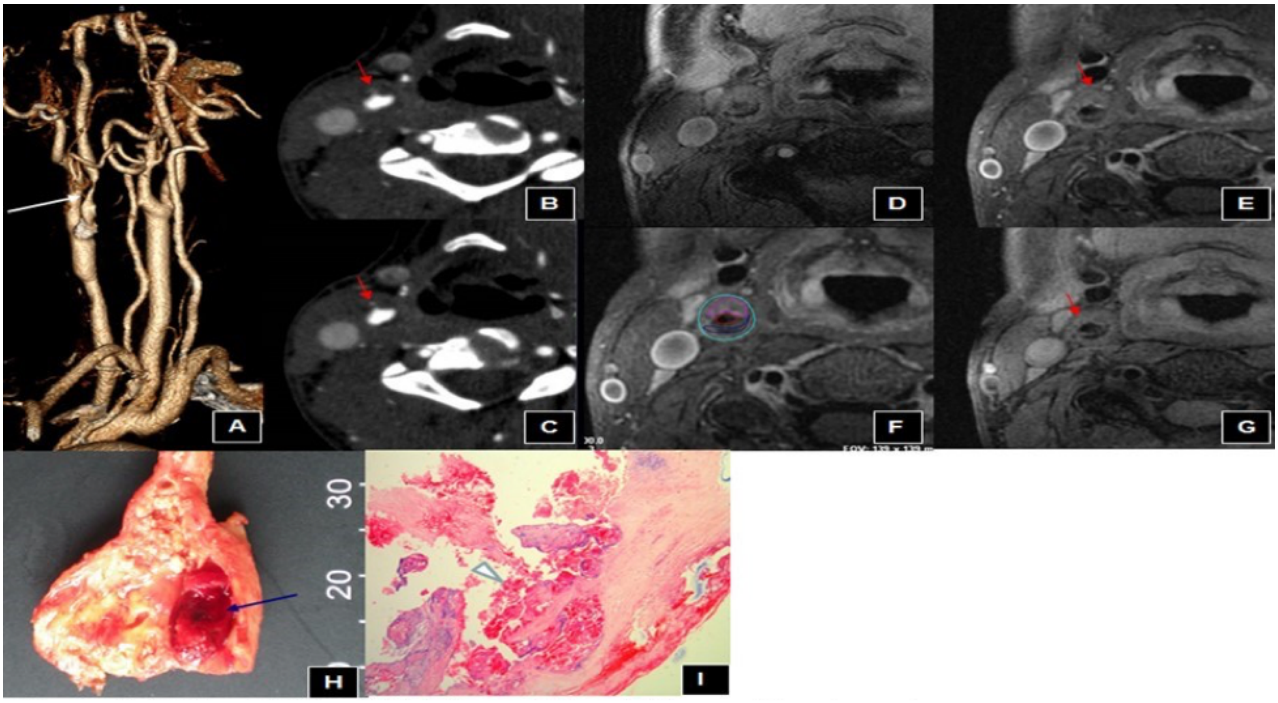


**FIGURE 2.** CTA, MRI, AND HISTOLOGY OF THE RIGHT CAROTID ARTERY PLAQUE.

A, CTA showed 90% stenosis in the bifurcation of the CCA and proximal ICA (white arrow); B-C, two transverse slices of the CTA showing a low-density plaque in the R-CCA (red arrow); D-G, MR, TOF, T1 W, T2W, and PD images of the R-CCA (direction and location same as in B). Red arrows indicate plaques: the pink contour shows thrombosis with an irregular surface; H, histology showed red thrombosis on the plaque surface; I, specimen analysis confirmed the rupture, subsequent thrombus formation, and calcification (arrowhead).

**TABLE 1.**

| Time | Events |
|---|---|
| Initial presentation | Intermittent blurred vision of the right eye, headache, nausea, and vomiting, for the past 8 years without obvious causes. |
| Day 1 | Transfer to ultrasound department for carotid color Doppler ultrasound, intracranial Doppler ultrasound, which showed bilateral common carotid artery (CCA), internal carotid artery (ICA), and external carotid artery (ECA) plaque with >70% stenosis. Right intracranial ICA was nearly occluded, with high resistance and low blood flow. |
| Day 2 | Transfer to the radiology department for computed tomography carotid angiogram, which confirmed mix plaque with plate-like calcification located at the bifurcation of the right CCA, proximal ICA, and ECA. lumen stenosis >90% |
| Day 4 | Transfer to the radiology department for high-resolution MRI. Carotid artery wall imaging showed plaque located at the right bifurcation of the CCA extended to the ICA, lumen stenosis close to occlusion, plaque surface ulcer-like with thrombosis. Lumen stenosis >90% |
| Day 15 | Right carotid artery endarterectomy confirmed yellow fatty-like plaque with thrombosis at the bifurcation of the CCA |
| 1 Month | Carotid color Doppler ultrasound showed normal blood flow of the right carotid artery and internal carotid artery, without lumen stenosis. |
| Outpatient clinic (6 months) | Marked improvement in patient's symptomatic status, no intermittent blurred vision onset |

surface coils (Shanghai Chenguang Medical Technologies CO, LTD, Shanghai, China). A standardized protocol was used to obtain three different contrast-weighted images (time of flight [TOF], and T1-, T2-weighted images) of the carotid arteries 2 cm proximal and 2 cm distal to the bifurcation, respectively. MRI of the carotid showed bilateral CCA, ICA, and ECA wall thickening, as well as diffused plaque formation. Plaque signals of the left side showed iso-intensity in TOF, T1w and T2w sequences. Calcification was reflected by hypo-intense areas located at the middle layer of the plaque, with moderate stenosis. The plaque located at the right bifurcation of the CCA extended to the ICA, causing lumen stenosis close to

occlusion. Notably, the plaque surface was irregular, showing ulcer-like changes with slightly high signals in T1w and iso-intensity in TOF and T2w sequences (Fig. 2 D-G). Based on our clinical experience, we considered these as vulnerable plaques, which may be associated with ulcers and thrombosis.

The plaque characteristics, combined with the clinical presentation of the patient, prompted us to perform right carotid artery endarterectomy. Gross histology revealed plaque components were yellow fatty-like matter; moreover, there was thrombosis at the bifurcation of the CCA, which easily dropped from the plaque. The thrombosis was about 10mm long, with an irregular surface like ulcers (Fig. 2 H). Serial cross-sections with a thickness of 10 μm or less were obtained at 0.5 mm intervals from each plaque specimen. These sections were stained with hematoxylin and eosin and examined microscopically by a pathologist blinded to the MRI results. Histology confirmed plaque rupture, subsequent thrombus formation, and calcification. Thus, the vulnerability of the plaque, which was suspected based on the MRI results, was confirmed through histologic examination. At 1-month follow-up, carotid color Doppler ultrasound showed normal flow and no lumen stenosis of the right carotid artery and internal carotid artery blood. At six months follow-up, the patient did not report a recurrence of her symptoms.

## DISCUSSION

Patients with FH are at very high risk of developing atherosclerosis at a young age. Increased carotid intima-media thickness (IMT) and carotid plaques may be apparent in FH patients already during childhood and (especially in HoFH) may lead to clinical events before reaching adulthood, as in the patient described in our case report. Carotid plaque burden may be assessed by carotid ultrasonography[8]. More detailed characterization of plaques in HoFH patients may be useful for risk stratification and as an outcome measure in clinical studies. However, very few studies have been done on assessing plaque composition in patients with FH, especially in HoFH[9]. High-resolution MRI of the carotid artery has the ability to qualitatively and quantitatively assess the main components of advanced human carotid atherosclerotic plaques in vivo and was found to correlate with findings of histologic plaques[10]. MRI-based quantification is accurate and reproducible and has, therefore, been used as an endpoint in clinical studies of anti-atherosclerotic therapies. However, previous studies were mainly focused on the plaque burden in the aorta wall in asymptomatic patients with heterozygous FH by using MRI[11-13]. Therefore, our data on carotid plaque burden may provide some information to the patients with HoFH.

## CONCLUSION

Our case demonstrates that high-resolution multi-contrast MRI played an excellent role in identifying carotid plaque components in a patient with HoFH, which can help clinical physicians reach a diagnosis and provide an intervention earlier so as to minimize the risk of developing atherosclerotic cardiovascular disease and related complications.

### List of abbreviations

HoFH = Homozygous familial hypercholesterolemia
ACVD = atherosclerotic cardiovascular disease
PCI = percutaneous coronary intervention
CCA = bilateral common carotid artery
ICA = internal carotid artery
ECA = external carotid artery
MR = magnetic resonance
TOF = time of flight
T1w = T1-weighted
T2w = T2 weighted
IMT = intima-media thickness

### Declarations

Ethical Approval and Consent to participate

The study has been approved by the Beijing AnZhen Hospital ethics review board. The design is reasonable, data collection standardized, and the relevant rights and interests of the patients have been fully protected.

### Consent for publication

Not applicable.

### Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

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## Competing interests

There are no conflicts of interest.

## Authors' contribution

Conception and design: Zhenjia Wang and Wei Yu. Collection and assembly of data: Zhenjia Wang and Wen Liu. Data analysis and interpretation: Zhenjia Wang, Long Jiang, and Luya Wang. Manuscript writing: Zhenjia Wang and Wei Yu. Revised the language/article and final approval of manuscript: all authors.

**RESUMO**

*A hipercolesterolemia familiar homozigótica, uma doença patogênica rara do metabolismo da lipoproteína intimamente relacionada com a doença cardiovascular aterosclerótica prematura, pode conduzir a uma elevada deficiência e mortalidade. A hipercolesterolemia familiar homozigótica afeta tipicamente não só a raiz aórtica, comprometendo os óstios coronários, mas também outros territórios, como a carótida, a aorta descendente e as artérias renais. Imagens de ressonância magnética multicontraste de alta resolução (RM) fornecem um método validado e útil para caracterizar quantitativamente as placas de aterosclerose da artéria carótida. No entanto, muito poucos estudos foram feitos sobre a avaliação da composição da placa em doentes com hipercolesterolemia familiar homozigótica utilizando ressonância magnética de alta resolução. Este trabalho deve avaliar o valor da ressonância magnética no acesso à doença da artéria carótida em doentes com hipercolesterolemia familiar homozigótica. Descrevemos um paciente de 28 anos de Pequim, China, que se apresentou à clínica neurológica com visão turva intermitente do olho direito, dor de cabeça, náuseas e vômitos por oito anos sem causas aparentes. Suspeitava-se de hipercolesterolemia familiar com base no histórico médico e no exame laboratorial. O ultrassom Doppler carotídeo mostrou uma artéria carótida bilateral comum, artéria carótida interna e parede da carótida externa espessando-se com sinais hiperecoicos. Posteriormente, a ressonância multicontraste de alta resolução da carótida mostrou calcificação com áreas hipointensas localizadas na camada média da placa, com estenose moderada. A placa localizada na bifurcação direita da artéria carótida comum estendia-se até a artéria carótida interna, causando estenose do lúmen próxima à oclusão. O paciente foi tratado com endarterectomia da artéria carótida direita. Em seis meses de acompanhamento, não houve recorrência dos sintomas do paciente.*

PALAVRAS-CHAVE: *Espectroscopia de ressonância magnética. Placa aterosclerótica. Espessura íntima-média da carótida. Artérias carótidas. Hiperlipoproteinemia tipo II.*

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ORIGINAL ARTICLE

# Dosages of androgenic hormones in adolescent patients with severe acne

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## SUMMARY

OBJECTIVE: *Acne vulgaris in female adolescents, when severe or accompanied by other signs of androgenization, may represent a sign of hyperandrogenemia often underdiagnosed, which will have harmful consequences for adult life. The objective of this cross-sectional and retrospective study was to demonstrate the incidence of hormonal changes in the cases of female adolescents with severe or extensive acne, with or without other signs of hyperandrogenism, and propose a hormonal research pattern which should be indicated in order to detect early hyperandrogenemia.*

METHODS: *The medical records of 38 female patients aged between 9 and 15 years old with grade II and/or III acne were analyzed. The dehydroepiandrosterone sulfate, dehydroepiandrostenedione, and androstenedione, total testosterone, and dihydrotestosterone sulfate hormones were required prior to initiation of treatment. The hormonal dosages were performed in the serum after at least 3 hours of fasting by means of radioimmunoassay tests.*

RESULTS: *Of the 38 patients included, 44.7% presented changes in androgen levels (hyperandrogenemia), and the two most frequently altered hormones were DHEA and androstenedione, with the same incidence (23.6%).*

CONCLUSIONS: *The correct and early diagnosis provides an effective and agile approach, including antiandrogen therapy, with the purpose of avoiding the reproductive and metabolic repercussions, besides controlling the inflammatory picture and avoid aesthetic complications.*

KEYWORDS: *Acne vulgaris. Adolescent. Androgens. Hyperandrogenism.*

## INTRODUCTION

The human skin is the target of a large number of chemical messengers, among which there are many hormones. The hormonal effect on the development of secondary sexual characteristics from puberty and the capacity of the pilosebaceous unit for producing and releasing hormones is already known. Hormones, among them the steroids, biologically affect the skin through high-affinity receptors in both nuclear and cytoplasmic membranes[1].

When adolescence starts, there is a physiological elevation of serum levels of androgens in both sexes[2]. The hormonal trigger, and the resulting increase in

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the androgen production, especially the increase in DHEAS levels (dehydroepiandrosterone sulfate), a testosterone precursor[3,4], acts on the sebaceous glands and promotes the excessive production of sebum, leading to the abnormal detachment of keratinocytes and the obstruction of the follicle opening, with consequent formation of the microcomedo[5], which is considered the initial stage of subclinical acne lesions[6]. The accumulation of sebum and the modification of its composition in the sebaceous gland favor the proliferation of gram-positive bacteria *Propionibacterium acnes* in genetically predisposed individuals[5]. This, in turn, leads to the release of cytokines such as interleukins IL-6 and IL-8 by the infundibular keratinocytes and IL-8 and IL-12 by macrophages, as well as pro-inflammatory mediators, resulting in the induction of inflammatory signaling in the pilosebaceous unit, which is an essential component in the initial process of acne lesions[7,8].

Thus, the key factors involved in the acne pathogenesis are classically summarized as 1) androgen hormone trigger, 2) sebaceous hypersecretion, 3) follicular hyperkeratosis with microcomedo formation, 4) proliferation of *P. acnes,* and 5) resulting inflammatory response[9,10].

Acne vulgaris is one of the most common pathologies in dermatology clinics, and a study with adolescents aged over 16 years in New Zealand estimated that 91% of boys and 79% of girls suffer from acne[11]. It can be observed at any age, but is more prevalent and more severe during puberty, with peaks of prevalence at 14-17 years of age in women and at 16-19 years in man. It tends to resolve spontaneously between the age of 20-25 years in both sexes, although this age is being prolonged[12]. It mainly affects the face, trunk, and back, with injuries ranging from comedos to inflammatory nodules[13].

In this context, the presence of acne in female adolescents aged between 12 and 17 years is expected. However, when it presents as a severe and extensive condition accompanied by other signs of androgenization, such as hirsutism and/or an increase of fat deposition in the abdominal subcutaneous tissue, i.e., with a male pattern of deposition with or without weight gain[14], the possibility of increased serum levels of androgens should be contemplated. Since the situation described is not always valued by dermatologists, leading to underdiagnosed cases of hyperandrogenism, many of these patients carry its harmful consequences into adult life, such as a body with a male pattern, acne scars, and hirsutism.

The objective of this retrospective cross-sectional study is to demonstrate the incidence of hormonal changes in cases of female adolescents, aged between 9 and 15 years, with severe or extensive acne, accompanied or not by other signs of hyperandrogenism, and propose a hormonal investigation pattern action that should be indicated for the early detection of a hyperandrogenemia scenario, avoiding its future aesthetic and metabolic manifestations.

## METHOD

### Patients

We analyzed the medical records of female patients treated in the outpatient clinic of Acne in Adult Women of the Dermatology Discipline of FMABC between 2010 and 2016. We included patients aged between 9 and 15 years old with papular-pustular acne (grade II) or cystic acne (grade III) affecting the face, with over 15 inflammatory lesions at the time of examination, with or without acne scars, with or without hirsutism and hypertrichosis. We excluded patients aged below 9 above 15 years old with acne grade I, grade II with less than 15 lesions at the moment of the examination and without acne scars, as well as patients in use of topical or systemic corticosteroids, vitamin supplements, anticonvulsants, and hormonal treatments, including anabolic steroids. This study was approved by the Research Ethics Committee of the Faculty of Medicine of ABC (87780418.5.0000.0082). All the people responsible for the participants read and signed the Informed Consent Form.

### Hormonal Dosage

The dehydroepiandrosterone sulfate (DHEAS), dehydroepiandrosterone (DHEA), androstenedione, total testosterone, and dihydrotestosterone (DHT) were systematically requested in the first consultation, before the initiation of treatment. The serum hormonal measurements were performed after at least 3 hours of fasting by radioimmunoassay examinations. The measurement methods used followed the international standardization for each of the respective hormones and the good practices; the values obtained are considered in accordance with the standards recommended in the guidelines for clinical analysis[15].

## RESULTS

We analyzed the medical records of 38 female patients aged between 9 and 15 years old, with acne grade II and/or III affecting the face; 23 patients also had lesions on the trunk and/or back (Figure 1). The onset of the lesions occurred between the ages of 9 to 15 years (mean of 12.8 years). The analysis showed that 17 patients (44.7% of the total) had high results in at least one of the hormones measured (the distribution is described in Table 1) in an isolated way or with combined high levels; 9 patients (23.6%) had more than one abnormal results, and DHEA and androstenedione was the combination more frequently observed (five patients). It should be noted that 23 patients (60.5% of the total) had hypertrichosis or hirsutism associated, based on the Ferriman-Gallway scale. Of the total number of patients, 17 reported menstrual irregularities (44.7%). Of the 12 patients who had the onset of symptoms at ages 14 or 15 years, i.e., two or more years after menarche, five had hormonal abnormalities and four, menstrual irregularities.

Of 38 medical records analyzed, 25 contained ultrasounds of the pelvic region; 17 patients (68%) presented polycystic ovaries upon examination.

For the diagnosis of polycystic ovary syndrome (POS), according to the Rotterdam consensus, two of the following data must be taken into account: hyperandrogenemia or hyperandrogenism, menstrual irregularities, and polycystic ovaries found on ultrasound. Thus, among the 38 patients studied, 23 (60.5%) presented the criteria for a POS diagnosis[16].

It should be noted that for patients with hyperandrogenemia and normal menstrual cycles, serum 17-OH (17-hydroxy-progesterone) measurement was subsequently requested to evaluate the possibility of benign adrenal hyperplasia. Two patients presented high values, thus being diagnosed with the condition.

**FIGURE 1.** A 12-YEAR-OLD PATIENT WITH EXTENSIVE CYSTIC ACNE ON THE FACE (A) AND BACK (B).

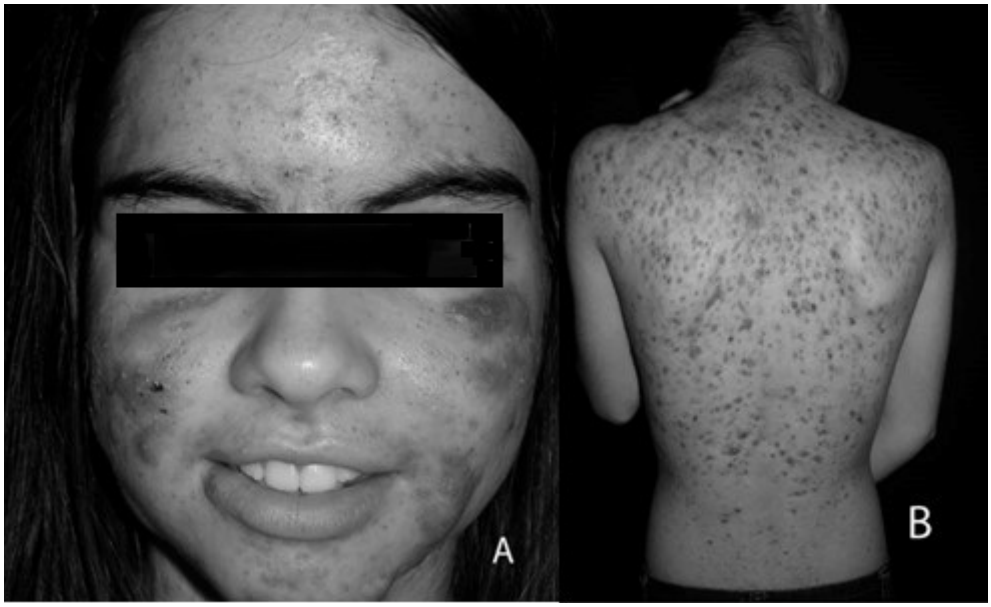


**TABLE 1.** NUMBER OF PATIENTS WITH ABNORMAL HORMONE LEVELS (N) AND PERCENTAGES BASED ON THE TOTAL NUMBER OF PATIENTS AND PATIENTS WITH ABNORMAL HORMONAL LEVELS. DHEA = DEHYDROEPIANDROSTERONE; DHT = DIHYDROTESTOSTERONE ; DHEAS = DEHYDROEPIANDROSTERONE SULFATE.

| Hormones | n | % of total patients (total of patients= 38) | % among patients with abnormal hormonal level (total of patients with abnormal levels =17) |
|---|---|---|---|
| DHEA | 9 | 23.6% | 52.9% |
| Androstenedione | 9 | 23.6% | 52.9% |
| Testosterone | 4 | 10.5% | 23.5% |
| DHT | 4 | 10.5% | 23.5% |
| DHEAS | 2 | 5.26% | 11.7% |

## DISCUSSION

Acne is a fairly common complaint by female patients in dermatological clinics and remains a therapeutic challenge[11]. When adolescence starts, there is a physiological elevation of serum levels of androgens in both sexes[2]. These hormones promote the hypersecretion of sebaceous glands, the hyperkeratinization of the follicular ostium, and the modification of the sebum composition, influencing the proinflammatory events that are characteristic of acne vulgaris. In females, however, from the moment after ovulation is completely established, approximately two years after menarche, the estrogen levels promote the improvement of acne[9].

A survey conducted with 2,895 women aged between 10-70 years of age showed that the peak incidence is in adolescence, being considered a disease characteristic of this stage of life[11]. However, when the acne is severe or extensive, in addition to greatly affecting the quality of life of patients, it may be a manifestation of hyperandrogenemia which, if left undiagnosed, can bring many damaging consequences during the adulthood of these patients, from an aesthetic perspective, because the inflammatory lesions and their potential to leave scars cause a significant social, educational, and functional impact, regardless of its severity[17], as well as from a metabolic perspective[9].

Although androgens are related to the pathogenesis of acne, articles that correlate the elevation of its serum levels in women of childbearing age and acne are inconsistent in the literature, ranging from 30% to 90% in some samples of patients, with an absence of changes in others[3,18]. Cunha et al.[9], in a study with 835 patients, concluded that the serum levels of DHEA androgens, androstenedione, and testosterone, DHT and DHEAS must be part of a hormonal assessment in women above 15 years of age with acne, and that DHEA is the androgenic hormone with most frequently high levels. However, the study did not evaluate patients with severe acne with the onset of lesions between 9 and 15 years of age, as presented in this work.

The results obtained in this study showed that 44.7% of the total number of patients presented changes in the levels of androgens (hyperandrogenemia) and that the two exams that have abnormal results more frequently were the DHEA and androstenedione, with the same incidence (23.6%). Cunha et al.[9], in turn, found 56.5% of patients with androgenic changes, and 21.3% had changes in DHEA.

Hyperandrogenism plays an essential role, affecting approximately 5-10% of women of childbearing age worldwide[19]. Among the diseases that promote signs of increased androgen production in adult women, the most common is polycystic ovary syndrome (POS), which affects approximately 70-72% of the patients, followed by idiopathic hyperandrogenism in 15%, idiopathic hirsutism in 10%, and non-classic congenital suprarenal hyperplasia in 3%. The estimated prevalence of disorders due to the excess of hormones in the general population is only possible for POS, and it varies between 4% and 14% in the female population since the prevalence of others, such as idiopathic hyperandrogenism or idiopathic hirsutism, unknown[20]. The clinical manifestations and laboratory findings in adolescents and adult women with POS and HCBSR share similarities. However, the differential diagnosis is essential to ensure that the therapeutic options can be individualized for each patient according to their needs[21].

On the other hand, the POS diagnosis in adolescents remains controversial, and experts continue to discuss the appropriate diagnostic criteria[22-26]. The ideal age for POS investigation is still not established[23,] and several studies have shown that the incidence in adolescents is high and underdiagnosed[26]. Among the studies presented, Hickey et al.[23] propose the age of 15.2 years as the threshold age, whereas Glueck et al.[24] established it as 14 years old in their work. Bronstein et al.[22], in turn, conducted a study in two populations: children under 13 years old and between 13-18 years to establish the age for diagnosis. They concluded that in patients with early puberty, 13 years old (or 1.5 years after pubarche and thelarche) should be considered in patients with clinical signs of hyperandrogenism.

In this study, the average age of lesion onset was 12.8 years, with one 9-year-old patient who met the inclusion criteria and, according to the criteria recommended by the Rotterdam consensus, 60.5% of the patients met the criteria for the POS diagnosis. Therefore, more important than the age of onset, the clinical condition of the patient must be considered; patients with severe acne, with or without evident signs of hyperandrogenism, must have their hormone levels evaluated to determine the best therapeutic conduct, which should be instituted as early as possible in order to avoid harmful manifestations in the future.

## CONCLUSIONS

The importance of a hyperandrogenemia diagnosis in cases of severe acne affecting patients between the ages of 9 and 15 years should be emphasized. The correct and early diagnosis provides an effective and agile approach, including antiandrogen therapy, to avoid reproductive and metabolic effects, in addition, to control the inflammatory process and avoid aesthetic complications, such as scars and dyschromia, improving the quality of life and reducing the morbidity and mortality in this group of patients.

### Contribution of the authors

GC, CDMF, FLAF: Study design, data acquisition; CM, GC, RFS, SIFR: Data collection, analysis, and interpretation. All authors participated in the drafting, editing, and approval of the final version of the manuscript.


## RESUMO

OBJETIVO: *A acne vulgar em adolescentes do sexo feminino, quando grave ou acompanhada de outros sinais de androgenização, pode representar um sinal de hiperandrogenemia muitas vezes subdiagnosticado, que acarretará consequências danosas para a vida adulta. O objetivo deste estudo transversal e retrospectivo foi demonstrar a incidência das alterações hormonais nos casos de adolescentes do sexo feminino com acne grave ou extensa, acompanhada ou não de outros sinais de hiperandrogenismo e propor um padrão de pesquisa hormonal que deve ser indicado com o intuito de detectar precocemente o quadro de hiperandrogenemia.*

MÉTODOS: *Foram analisados os prontuários de 38 pacientes do sexo feminino com idades entre 9 e 15 anos, portadoras de quadro de acne grau II e/ou III. Os hormônios sulfato de dehidroepiandrostenediona, dehidroepiandrostenediona, androstenediona, testosterona total e dehidrotestosterona foram solicitados antes do início do tratamento. As dosagens hormonais foram realizadas no soro após pelo menos 3 horas de jejum por meio de exames de radioimunoensaio.*

RESULTADOS: *Das 38 pacientes incluídas, 44,7% apresentaram alterações dos níveis de andrógenos (hiperandrogenemia), sendo que os dois hormônios mais frequentemente alterados foram o DHEA e androstenediona, com a mesma incidência (23,6%).*

CONCLUSÕES: *O diagnóstico correto e precoce propicia uma abordagem efetiva e ágil, incluindo a terapia antiandrogênica, com a finalidade de evitar as repercussões reprodutivas e metabólicas, além de controlar o quadro inflamatório e evitar complicações estéticas.*


PALAVRAS-CHAVE: *Acne vulgar. Adolescente. Androgênios. Hiperandrogenismo.*

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ORIGINAL ARTICLE

# ADAMTS4 is upregulated in colorectal cancer and could be a useful prognostic indicator of colorectal cancer

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## SUMMARY

OBJECTIVE: *ADAMTS4 is a member of the ADAMTS4 family, which secretes proteinases. The mechanism of tumor metastasis may be correlated to its promotion of angiogenesis. It was determined whether ADAMTS4 participates in colorectal cancer progression. Methods: The expression in clinical samples and CRC cell lines was investigated. Using immunohistochemistry (IHC), fluorescence in situ hybridization (FISH), and RT-PCR, the expression of ADAMTS4 was determined in colorectal tumors of different cancer stages and anatomic sites, and in three cell lines of different aggressiveness. Results: The overexpression of ADAMTS4 was observed in tissue samples by IHC, and this was mainly located in the cytoplasm, as detected by FISH. The qRT-PCR and western blot analyses further supported the clinical sample findings. Conclusion: The present data support the notion that the overexpression of ADAMTS4 in CRC might be useful as a non-invasive biomarker for detecting CRC in patients.*

KEYWORDS: *Neoplasms. Neoplasm Metastasis. ADAMTS4 Protein.*

## INTRODUCTION

Colorectal cancer (CRC) is common worldwide and remains as the leading cause of cancer-related deaths in Western countries, as well as in the rest of the world.[1] More than 1.2 million new cases of CRC occur every year globally.[2] Although screening strategies have improved and more effective treatments have been developed, the prognosis of advanced CRC remains poor. Hence, developing novel diagnostic or therapeutic biomarkers remains as an urgent necessity.

Disintegrin and metalloproteinase with thrombospondin motifs 4 (ADAMTS4), which is capable of cleaving aggrecan, brevican, neurocan, and versican, is an enzyme encoded by the *ADAMTS4* gene,[3] and is also encoded as a member of the ADAMTS protein family. Previous studies have shown that ADAMTS4 is involved in cartilage destruction in human rheumatoid arthritis and osteoarthritis.[4,5] Moreover, ADAMTS4 may also contribute to the progression of central nervous system disorders.[6,7] In cancers, ADAMTS

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can play both oncogenic and tumor-protective roles by regulating matrix-degradation, angiogenesis, and metastasis.[8,9]

Extracellular matrix (ECM) components are critically involved in the malignant phenotypes of cancers, especially metastatic events. Specifically, the protease-induced breakdown of ECM components is essential for tumor cells to cross tissue barriers.[10,11] However, the functions, mechanisms of activation, and substrates of most ADAMTS members remain largely unexplored, especially in CRC. Therefore, exploring the roles of ADAMTS in CRC might provide promising oncotherapeutic options. In the present study, the expression profile of ADAMTS4 in CRC clinical specimens was studied first. Based on the finding that ADAMTS4 was highly expressed in CRC tissues, the following were further investigated: (1) the correlation between ADAMTS4 and clinicopathological factors; (2) the expression of ADAMTS4 in CRC cell lines.

## MATERIALS AND METHODS

### Studies on human patient tissues

Cancerous colon tissues and adjacent normal tissues were obtained from patients (41 patients, mean age; 66 years old, age range; 46–78 years old) who underwent a surgical operation due to CRC. Among the patients included in the present study, six were at stage A (Duke's), 12 were at stage B, 14 were at stage C, and nine were at stage D. All patients included in the study were free of other diseases and never suffered from any disease prior to the study. The study design was approved by the Ethics Committee of the Second People's Hospital of Yunnan Province, China, and written informed consent was obtained from each participant.

### Immunohistochemistry

A 5-μm section was cut and mounted on a poly-L-lysine coated slide for routine histological immunohistochemistry and bare slides. Then, the tissue sections were separated in xylene and supplemented with graded ethanol. Afterward, these were incubated with 3% hydrogen peroxide, placed in a microwave for 15 minutes to remove the antigenic epitopes, and blocked with 10% BSA (Bioteke, Beijing, China). After washing three times with phosphate-buffered saline (PBS), the sections were incubated with the primary antibody for ADAMTS4 (Abcam, ab185722; diluted at 1:300) at 4°C overnight with optimal dilution. The next day, the sections were incubated with the second antibody. Two experienced pathologists individually reviewed the immunohistochemistry (IHC) studies. They agreed for most of the cases (>80%), and the others were discussed until they arrived at a consensus. ADAMTS4 IHC scoring was performed using these two scoring criteria[12]: The first criteria were based on the intensity of the staining: 0 = no staining; 1 = weak staining; 2 = moderate staining; 3 = strong staining. The second criteria were based on the proportion of stained cells: 0 = staining of 1-10%, 1 = 11-25%, 2 = staining of 26-50%, 3 = staining of 51-75%, 4 = staining of more than 76%. The total score was calculated by adding the points for intensity and proportion of stained cells. The maximum score was 7, and the minimum was 0. A score of 0-1 was considered weak, a score of 2-4 moderate, and 5-7 was considered overexpression. These were used to categorize various histological types.

### Fluorescence in situ hybridization

In order to detect ADAMTS4 in clinical specimens, fluorescence *in situ* hybridization (FISH) was used, according to a previously described method[13,14], with some modifications. For paraffin-embedded tissues, after deparaffinization and rehydration, the samples were treated with a peroxidase-quenching solution. Proteinase K was added to digest tissues before prehybridization and hybridization, which were carried out at 55°C for 30 minutes and four hours, respectively. These slides were fixed in RiboFix and counterstained with 4'-6'diamidino-2-phenylindole (DAPI) in an antifade reagent (Ventana). The images were acquired using a Nikon A1RVAAS laser point- and resonant-scanning confocal microscope, with an excitation of 488 nm for FITC.

### Cell culture

The normal colonic epithelial cell line NCM460 was a kind gift from Kunming Bacon Biotechnical Company. Three human CRC cell lines (SW116, Caco-2, and HCT29) were obtained from the Cell Bank of Type Culture Collection of the Chinese Academy of Sciences in Shanghai, China. Cells were cultured in Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal bovine serum (FBS). All cells were cultured in a humidified atmosphere with 5% CO2 at 37°C.

### RNA isolation and quantitative real-time PCR

For the real-time PCR analysis, RNA was isolated from CRC cells using Trizol reagent

(Invitrogen, Carlsbad, CA, USA), and 1 µg of RNA was reverse-transcribed into cDNA using a PrimerScript RT-PCR kit (Takara, Tokyo, Japan), according to manufacturer's protocol. The SYBR-Green PCR master mix (Applied Biosystems, CA, USA) containing 333 nM of each forward (F) and reverse (R) primer, and 2.5 µL of the reverse-transcribed template were transferred into a 96-well PCR plate (Beaver, Suzhou, China). The primer used was as follows: human ADAMTS4 forward, 5'-GAGGGAGGCACCCCTAACT-3'; ADAMTS4 reverse, 5'- CCTTGACGTTGCACATGGGA-3'. PCR was performed in the Applied Biosystems 7300 Fast Real-Time PCR System. The melting curve analysis was used to monitor the specificity of the PCR products. GAPDH mRNA was used as an internal control.

### Western blot

The total proteins of CRC cells were extracted using RIPA lysis buffer (Cat#: 89900, Thermo Fisher). The protein lysates were separated by 10% sodium dodecyl sulfate-polyacrylamide gel (SDS-PAGE). After electrophoresis, the proteins were electrotransferred on nitrocellulose membranes (Solarbio, Beijing, China) using the following transfer buffer: 25 mM of Tris, 190 mM of glycine and 20% methanol. The protein transfer was performed at 50 V for one hour at 41°C. The blotted membranes were incubated in a blocking solution containing 5% (w/v) non-fat milk powder and 0.1% (v/v) Tween-20, for one hour at room temperature. Then, the membranes were incubated with the primary antibody of ADAMTS4 (Abcam, ab185722; diluted at 1:1,000) and GAPDH (Abcam, ab9485; diluted at 1:2,000) at 4°C overnight, followed by incubation with secondary horseradish peroxidase (HRP)-conjugate anti-rabbit secondary antibody for one hour.

### Statistical analysis

All data were presented as mean ± standard deviation (SD), unless otherwise not indicated. The differences between multiple groups were analyzed by one-way ANOVA analysis. Features considered as potential explanatory factors were as follows (reference category in italics): gender (male *vs.* female), age (<60 years old *vs.* >60 years old), Duke's stage (A, B, C and D), histological tumor type (mucinous *vs.* non-mucinous), and ADAMTS4 expression (weak ADAMTS4 expression *vs.* strong ADAMTS4 expression). $P<0.05$ was considered statistically significant for all tests. The statistical analyses were performed using the SPSS 16.0 software (SPSS, Chicago, IL, USA), and graphical representations were done using GraphPad Prism 5 (San Diego, CA, USA) software. The correlation of ADAMTS4 expression with clinicopathologic parameters was analyzed by the Pearson chi-square test.

## RESULTS

### ADAMTS4 is upregulated in tissues obtained from CRC patients

Based on previous studies and published research by the investigators, the focus was on the study of ADAMTS4 to its expression profile in CRC. IHC analysis was carried out to detect the ADAMTS4 protein expression both in CRC tumor tissues and the corresponding adjacent normal tissues of the 41 CRC patients. A higher expression of ADAMTS4 was detected in CRC tumor tissues when compared to that in adjacent normal tissues in 30 patients, accounting for 73.2% of all patients (Figure 1A). The combined average IHC score was 2.58 ± 0.23 in adjacent normal tissues and 4.34 ± 0.24 in tumor tissues, respectively (Figure 1B, $P<0.001$). The representative ADAMTS4 staining is presented in Figure 1C. Furthermore, the correlation between the expression of ADAMTS4 and the corresponding clinicopathological parameters of CRC patients was analyzed using Chi-square. As shown in Table 1, ADAMTS4 protein expression was both correlated with tumor size ($P=0.05$) and TNM stage ($P=0.005$), while no significant difference was found in age, gender, tumor location, and serum CEA level and histology. In order to further determine the mRNA expression of ADAMTS4, FISH was carried out, and it was found that the ADAMTS4 mRNA was located mainly in the cytoplasm (Figure 1D).

### ADAMTS4 mRNA is upregulated in CRC cell lines

In order to investigate the expression of the *ADAMTS4* gene, RNA was extracted from both benign and malignant cells. *ADAMTS4* expression was initially investigated in one normal and three malignant CRC cells, where it was observed that there was a significantly higher expression in malignant cells when compared to benign cells, NCM460 (2.25-, 4.47- and 1.68-fold increase in SW116, Caco-2, and HCT29 CRC cell lines; $P=0.002$; Figure 2A).

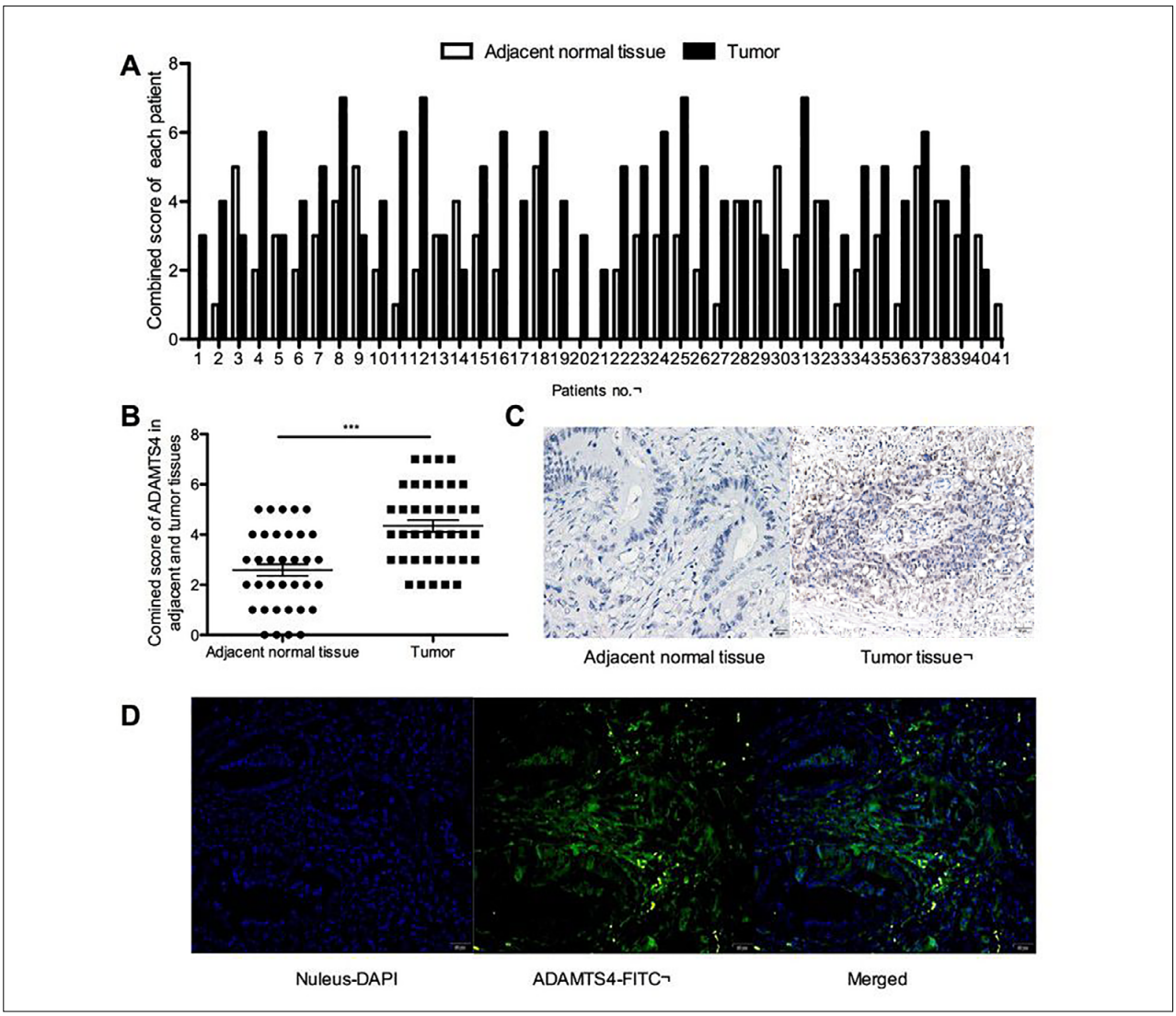


**FIGURE 1.** ADAMTS4 is upregulated in CRC clinical samples and mainly located in the cytoplasm. (A) The IHC analysis in 41 CRC patients revealed a higher expression of ADAMTS4 in CRC tumor tissues, when compared to adjacent normal tissues, in 30 patients, accounting for 73.2% of all patients. (B) The combined average IHC score was 2.58 ± 0.23 in adjacent normal tissues and 4.34 ± 0.24 in tumor tissues, respectively ($P$<0.001). (C) The representative ADAMTS4 staining was shown in CRC clinical and adjacent normal tissues. (D) Fluorescence *in situ* hybridization was carried out, and it was revealed that ADAMTS4 mRNA was located mainly in the cytoplasm. Scale bars: 50 μm.

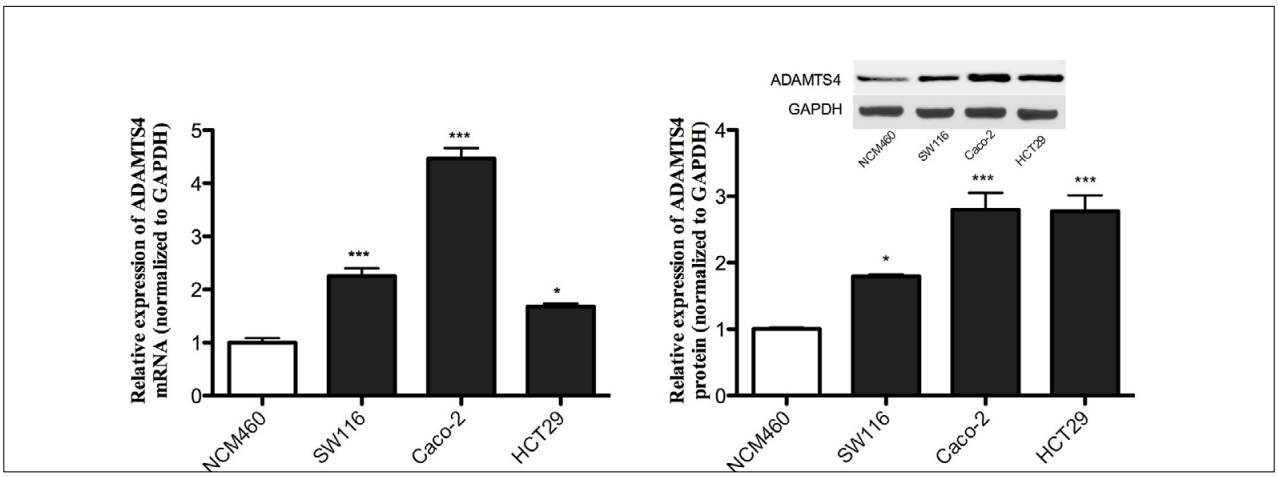


**FIGURE 2.** ADAMTS4 mRNA and protein are highly expressed in CRC cell lines. (A) The mRNA expression of ADAMTS4 increased in CRC cell lines when compared with normal NCM460 cells. (B) The western blot demonstrated that the protein expression of ADAMTS4 in CRC cell lines was highly upregulated. *$P$<0.05, **$P$<0.001.

### ADAMTS4 protein is upregulated in CRC cell lines

In order to verify the previous IHC and mRNA findings, the present study detected the protein expression of ADAMTS4 in the CRC cell lines. The protein expression of ADAMTS4 in CRC cells was significantly higher when compared with NCM460 cell lines (Figure 2B; 1.79-, 2.8- and 2.78-fold increase in SW116, Caco-2 and HCT29 CRC cell lines, respectively).

## DISCUSSION

In the present study, the expression of ADAMTS4 in CRC clinical specimens was first evaluated, and it was found that ADAMTS4 is highly expressed in CRC tissues when compared to adjacent normal tissues. Next, the mRNA distribution of ADAMTS4 was analyzed using FISH, and it was found that ADAMTS4 is mainly located in the cytoplasm. Finally, mRNA and protein expression of ADAMTS4 was detected in CRC cell lines and normal NCM460 cells, consolidating the previous findings that ADAMTS4 is highly expressed in CRC cells.

Previous studies have shown that ADAMTS1, ADAMTS2, ADAMTS5, ADAMTS8, ADAMTS9, and ADAMTS12 could play key roles in tumorigenesis and tumor angiogenesis.[15,16] ADAMTS4 has been mainly studied for its role in contributing to cartilage degradation in arthritic diseases.[17,18] In the present study, the new roles of ADAMTS4 in CRC progression were demonstrated, and ADAMTS4 was added as a new member of the ADAMTS family, which possibly influences tumor progression.

Previous studies have shown that an overexpressed profile of ADAMTS4 has been reported in several cancers, including glioblastoma, head and neck cancer, melanoma, and epithelial ovarian cancer.[8,9,19] Through the immunochemical analysis of 41 CRC tissues, it was confirmed that ADAMTS4 expression was elevated in CRC tissues, when compared to normal tissues, in CRC. Furthermore, according to the correlation between ADAMTS4 and the clinical characteristics, the overexpression of ADAMTS4 was identified as a crucial factor in CRC progression. These present results support the notion that ADAMTS4 is overexpressed in both CRC clinical specimens and CRC cell lines, which is consistent with previous studies.[20,21]

The present study had special advantages. In the beginning, the evidence was complete and consolidated. This was because ADAMSTS4 was detected in both clinical specimens and CRC cell lines, and the results were consistent. Next, the mRNA expression of ADAMTS4 was determined using FISH, and it was revealed that ADAMTS4 mRNA is mainly located in the cytoplasm, which possibly provides new insight into this molecule since a different location always implies a special functional approach.[22,23] However, there were some limitations in the present study. First, the number of patients recruited was too small, which may not be enough to draw these conclusions. Second, all patients came from a single center, which may not represent the true existence of ADAMTS4 in other ethical or regional CRC patients. Finally, the expression of ADAMTS4 was just detected in the CRC cell lines, without detecting the potential functional roles, such as the ability to potentially contribute to CRC cell proliferation, migration or invasion, among others. Hence, future studies should focus on further investigating the mechanisms of ADAMTS4 in CRC.

## CONCLUSION

The results presented in the present study provide both clinicopathological and cell level evidence that ADAMTS4 is overexpressed in both CRC clinical specimens and cell lines. Moreover, the correlation between ADAMTS4 expression and clinical characteristics demonstrates that highly expressed ADAMTS4 correlates with tumor progression in CRC.

### Authors' contributions

Xue-Qin Shang, Substantial contributions to the conception, design, and draft the work; 2) Xue-Qin Shang, Kui-Liang Liu, Qian Li, Yue-Qiong Lao, Nan-Shan Li, Jing Wu, acquisition, analysis, and interpretation of data for the work; 3) Kui-Liang Liu, Qian Li, Yue-Qiong Lao, Nan-Shan Li, Jing Wu, critical revision for important intellectual content; 5) Xue-Qin Shang, Kui-Liang Liu, Qian Li, Yue-Qiong Lao, Nan-Shan Li, Jing Wu, final approval of the version to be published; 6) Xue-Qin Shang, Kui-Liang Liu, Qian Li, Yue-Qiong Lao, Nan-Shan Li, Jing Wu, agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

## RESUMO

OBJETIVO: *ADAMTS4 é um membro da família ADAMTS4, que secreta proteinases. O mecanismo da metástase do tumor pode ser correlacionado a sua promoção da angiogênese. Determinou-se se ADAMTS4 participa na progressão do câncer colorretal. Métodos: A expressão em amostras clínicas e linhas de células CRC foi investigada. Usando a imuno-histoquímica (IHC), a hibridação fluorescente in situ (HFIS) e o RT-PCR, a expressão de ADAMTS4 foi determinada em tumores colorretais de diferentes estágios do câncer e locais anatômicos, e em três linhas de células de níveis de agressividade distintos. Resultados: A superexpressão de ADAMTS4 foi observada em amostras de tecido por IHC, e esta foi localizada principalmente no citoplasma, como detectado pelo HFIS. O qRT-PCR e a análise de wester blot corroboraram os resultados clínicos da amostra. Conclusão: Os dados atuais corroboram a noção de que a superexpressão de ADAMTS4 no CRC pode ser útil como um biomarcador não invasivo para a detecção de CRC em pacientes.*


PALAVRAS-CHAVE: *Neoplasias. Metástase neoplásica. Proteína ADAMTS4.*

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ORIGINAL ARTICLE

# Esophageal manometry in systemic sclerosis: findings and association with clinical manifestations

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## SUMMARY

INTRODUCTION: *Systemic sclerosis (SSC) is an autoimmune disorder that affects several organs of unknown etiology, characterized by vascular damage and fibrosis of the skin and organs. Among the organs involved are the esophagus and the lung.*

OBJECTIVES: *To relate the profile of changes in esophageal electromanometry (EM), the profile of skin involvement, interstitial pneumopathy (ILD), and esophageal symptoms in SSC patients.*

METHODS: *This is an observational, cross-sectional study carried out at the SSC outpatient clinic of the Hospital de Clínicas of the Federal University of Uberlândia. After approval by the Ethics Committee and signed the terms of consent, 50 patients were initially enrolled, from 04/12/2014 to 06/25/2015. They were submitted to the usual investigations according to the clinical picture. The statistical analysis was descriptive in percentage, means, and standard deviation. The Chi-square test was used to evaluate the relationship between EM, high-resolution tomography, and esophageal symptoms.*

RESULTS: *91.9% of the patients had some manometric alterations. 37.8% had involvement of the esophageal body and lower esophageal sphincter. 37.8% had ILD. 24.3% presented the diffuse form of SSC. No association was found between manometric changes and clinical manifestations (cutaneous, pulmonary, and gastrointestinal symptoms).*

CONCLUSION: *The present study confirms that esophageal motility alterations detected by EM are frequent in SSC patients, but may not be related to cutaneous extension involvement, the presence of ILD, or the gastrointestinal complaints of patients.*

KEYWORDS: *Systemic Sclerosis, Esophagus, Interstitial Lung Disease, Manometry*

## INTRODUCTION

Among collagen disorders, systemic sclerosis (SS) is a devastating disease with a profound impact on life expectancy and a probability of death 3.5[1] greater than the general population. Mortality varies according to the type of cutaneous involvement, diffuse or limited (more prevalent), and is more unfavorable in the diffuse form. The main cause of mortality in SS is cardiopulmonary involvement[1]. The most frequent pulmonary manifestation is interstitial lung disease (ILD), present between 57-86%[1] of these individuals. Gastroesophageal reflux is one of the mechanisms involved in ILD[2].

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The involvement of the gastrointestinal tract (GIT) is present in approximately 80% of individuals with SS[2]. The segment of the GIT more frequently involved is the esophagus, affected in 50-90% of patients with SS[3]. Due to the precocity of esophageal involvement even without classic symptoms, its presence should be investigated[4] since its consequences, such as bronchoaspiration, predispose pulmonary fibrosis, and Barrett's esophagus[5]. In addition, the symptoms of esophageal involvement (SEI), such as dysphagia, heartburn, and acid regurgitation, have a negative impact on the quality of life of patients with SS[6].

SEI may be absent in up to 50% of patients with involvement of the organ documented by complementary tests[4]. The investigation of the changes in esophageal motility is done mainly by esophageal electromanometry (EM)[4], which can find subtle alterations in the motility of the organ. In the ME, the scleroderma esophagus classically presents loss of motility of the distal third of the esophageal body (EB) and hypotonicity of the lower sphincter[7], but these changes are not always simultaneous nor exclusive of SS[8].

The medical literature describes the association between a higher frequency of esophageal abnormalities in patients with limited cutaneous involvement, as expressed by Leroy's criteria in 1988[9]. The association between SS esophagopathy and the diffuse cutaneous presentation of the disease, the severity of pulmonary damage[10], the pattern of the antinuclear factor, and specific antibodies[9,11] remains controversial[7].

The present study was performed in SS patients who attended the Clinical Hospital of the Federal University of Uberlândia (HC-UFU). Its objective is to correlate; in these individuals, the profile of ME changes with the type of cutaneous involvement with ILD and SEI.

## PATIENTS AND METHOD

### Patients

This is an observational, cross-sectional study conducted at the SS outpatient clinic of HC-UFU from December 2014 to June 2015, approved by the Ethics Committee (Platform Brasil, Opinion: 929.011). We invited to participate in the study patients of the Rheumatology Outpatient Clinic of HC-UFU aged over 18 years and with diagnosed SS according to the criteria of ACR/EULAR 2013[12]. The patients were separated based on the forms of cutaneous involvement, diffuse or limited, according to the criteria defined by Leroy[13].

The clinical and laboratory characterization was carried out based on the data obtained from the medical charts, which were as follows: sex, age, year of the first symptom of the disease, presence of pyrosis, dysphagia and reflux of gastric contents (SEI), Raynaud's phenomenon (RP), presence of anti-centromere antibody, and presence of the antibody anti-topoisomerase1, serology for Chagas Disease (two methods: hemagglutination and ELISA), echocardiogram, treatments with nifedipine, cyclophosphamide and bosentan, concomitance of diabetes and hypothyroidism.

In the analysis of the echocardiograms, pulmonary hypertension was considered when recorded in the examiner report and with measures of pulmonary artery pressure greater than 35 mmHg[14]. In the retrospective analysis, RP or thickening of the fingers noticed by the patient was considered as the first symptom of SS[15].

All 58 individuals with a SS diagnosis who attended the Rheumatology Outpatient Clinic of HC-UFU were invited to participate in the study; 50 agreed and signed the Informed Consent Form (ICF).

### Esophageal Manometry

All EMs were performed by the same gastroenterologist (Matoso, AGB). EM was performed, after a 6h fasting, with an electromanometer with a water perfusion catheter with four radial channels and four channels separated 5 cm apart (Alacer Bio-São Paulo). The normality criteria for the esophagus manometry findings were the following: Pressure of the upper sphincter of the esophagus (USE): 30-180 mmHg; lower esophageal sphincter (LES): 10-34 mmHg; Number of peristaltic waves: 8-10 (greater than 80%). LES hypotonia was defined when the pressure was lower than 10mmHg, and aperistalsis, as the absence of peristaltic function in the lower esophageal body (EB). Hypocontractility was characterized as more than 30% of waves not conducted and/or a mean amplitude in the distal esophagus of less than 30 mmHg[16].

### High-resolution Computed Tomography (HRCT)

Tomographic findings consistent with pulmonary impairment related to SS were considered the description of glass opacities, honeycombing of the parenchyma, or fibrosis in the lung bases in HRCT, and confirmed by the physician. Any one of these descriptions given by the radiologist was considered and related to SS. The HRCT was carried out in

several different services with reports from different radiologists[17].

### Statistical analysis

We performed a descriptive statistical analysis in percentages, means, and standard deviations to describe the sample. To assess the relationship between EM, HRCT, and esophageal symptoms (SEI), we used the Chi-square test. All analyses were performed using the SPSS software version 20.0. We considered a p-value < 0.05 to be significant.

## RESULTS

### General data of the population

Of the 50 patients included initially, only 37 underwent HRCT and EM and had their data analyzed in the present study. Of these, 30 (81.1%) are coming from Uberlandia. The mean age at the time of inclusion in the study was 50.22 years, with a minimum of 25 and a maximum of 70 years. The average time between the first manifestations of the disease and attending the specialized service was 5.2 years (SD of 8.48 years). Two individuals did not present the onset of the disease with RP. Other demographic data and clinical characterization are described in Table 1.

### Esophageal Manometry

Of the 37 EM patients studied, three had completely normal test results; therefore, 34 (91.9%) had some abnormality. There were 14 (37.8%) individuals with concomitant abnormalities in the EB and the LES. Fifteen (40.6%) patients presented exclusive involvement of the EB. Four patients had exclusive involvement of the LES: two had hypotonia, and two presented the LES hypertonia. In the series, we found two individuals with LES hypertonia concurrent with waves not conducted by the EB. One patient presented the upper sphincter with a tendency to low amplitude. In Figure 1, we can see a normal EM and, in figure 2, one often found among SS patients.

### High-resolution Computed Tomography (HRCT)

In the present study, 14 (37.8%) patients presented ILD, of these seven were anti- topoisomerase1 positive. In the group of ILD patients, seven (50%) presented diffuse cutaneous SS and the other seven (50%) the limited cutaneous presentation. In addition to ILD, there were seven patients with other findings, such as calcified nodules, atelectasis, blisters, and results compatible with pulmonary emphysema. Dilation of the Eb was described in four cases.

**FIGURE 1.** NORMAL CONVENTIONAL ESOPHAGEAL ELECTROMANOMETRY. PERISTALTIC CONTRACTIONS IN THE ESOPHAGEAL BODY (GREEN ARROWS), NORMOTONIC LOWER ESOPHAGEAL SPHINCTER WITH ADEQUATE RELAXATION DURING SWALLOWING (RED ARROWS).

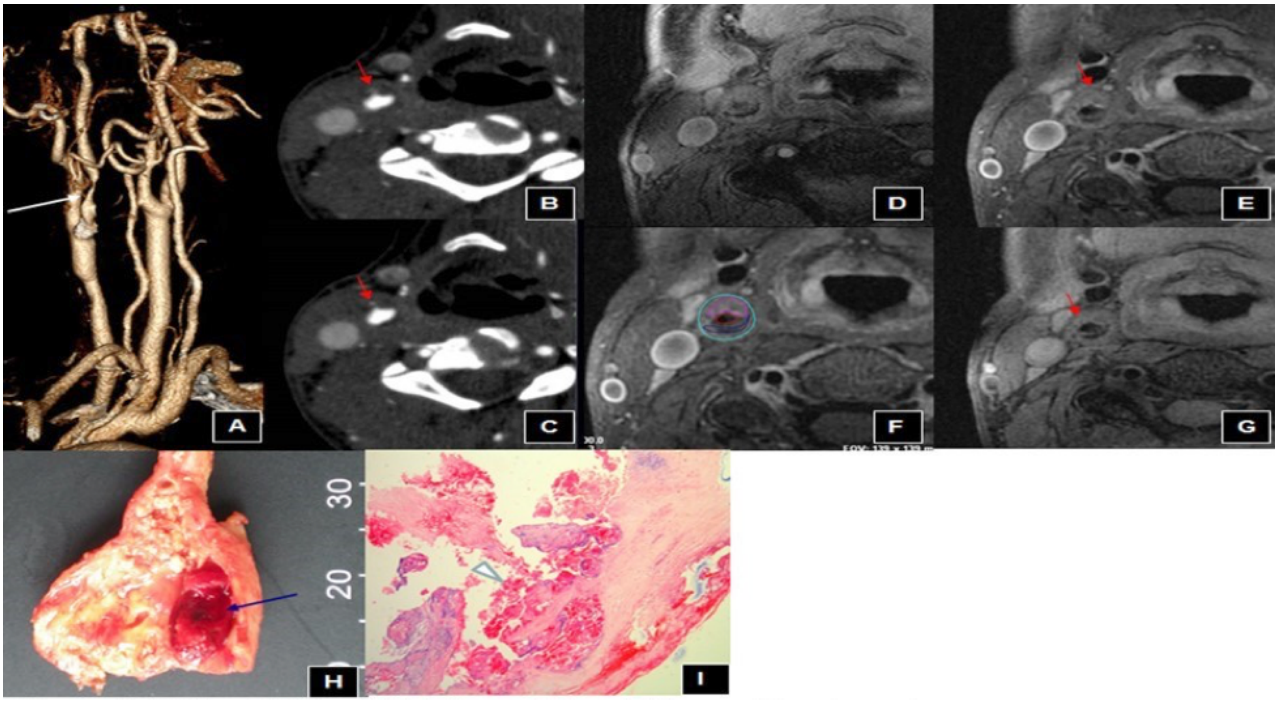

**FIGURE 2.** ESOPHAGEAL MANOMETRY IN A PATIENT WITH SYSTEMIC SCLEROSIS. HYPOCONTRACTILITY OF THE ESOPHAGEAL BODY WITH ABSENCE OF WAVES OF CONTRACTION (GREEN ARROWS), HYPOTONIA OF THE LOWER ESOPHAGEAL SPHINCTER WITH NORMAL RELAXATION DURING SWALLOWING (RED ARROWS).


The relationship between cutaneous involvement and the manometry findings

The distribution of the type of cutaneous involvement and the manometry findings were analyzed, and no significant difference was found in the frequency of abnormal EM in both groups, not even when the abnormalities of the EB or the LES (*p*:0.999) analyzed in isolation.

The relationship between pulmonary involvement and the manometry findings

No statistically significant correlation was found between manometry abnormalities exclusive to the EB or in association with LES, and ILD (*p*:0.736) were found.

The relationships of esophageal manifestations (ES), distribution by type of cutaneous involvement and EM and HRCT findings

Only 06 (16.21%) individuals did not present SEI. The relationship between abnormalities in the SEI and ES showed no statistical correlation; there were 34 patients with EM abnormalities and 31 with SEI (*p*: 0.70). The presence of SEI also did not correlate with the exclusive involvement of the EB, which totaled 15 (40.5%) cases, among which 13 (35.1%) had SEI (*p*: 0.087). There was also no statistically relevant correlation in the association of patients with SEI and individuals with hypotonia of the LES concomitant to the absence of waves in the EB; there were 12 (32.4%) symptomatic cases with that relationship, in comparison to the other 19 (51.3%) symptomatic patients with EM without the involvement of the LES (*p*: 0.152).

There was no statistically significant correlation between the presentation of cutaneous involvement and SEI; there were 23 (62.1%) individuals with limited cutaneous SS and symptomatic and 8 (21.6%) cases of diffuse cutaneous SS also symptomatic (*p*:0.154). The ones with SEI also did not correlate with the presence of ILD; there were 12 (32.4%) patients with symptoms and ILD, in comparison with 19 (51.3%) individuals with symptoms and without ILD (*p*: 0.999).

Among the patients who presented abnormalities in the EM, 15 (40.5%) were taking a calcium channel blocker, and 19 (51.3%) were not. Therefore, there was no relationship between the manometry findings and the use of calcium channel blockers (*p*: 0.678).

## DISCUSSION

The higher incidence of SS in females and greater prevalence of the limited cutaneous presentation are universal[18]. In this study, 92% of women had SS. The

**TABLE 1.** DEMOGRAPHIC, CLINICAL, AND IMMUNOLOGICAL DATA OF 37 PATIENTS WITH SS IN 2015.

| Variables and epidemiological data | Results (data are in numbers (%), except where indicated otherwise |
|---|---|
| Age at the onset of the disease (years), mean (± SD)<br>Female | 52.21 (12.06)<br>34 (92) |
| Form of cutaneous presentation<br>Limited | 28 (75.7) |
| Diffuse | 9 (24.3) |
| Esophageal symptoms (dysphagia, pyrosis, and/or regurgitation) | 31 (83.8) |
| Abnormal esophageal manometries | 34 (91.9) |
| Interstitial lung disease | 14 (37.8) |
| Pulmonary hypertension on echocardiogram | 5 (16.6*) |
| Comorbidities<br>Diabetes mellitus | 2 (5.4) |
| Hypothyroidism | 5 (13.5) |
| Anti-centromere antibody | 10 (27) |
| Anti-Topoisomerase I | 9 (24.3) |
| Medication<br>Use of cyclophosphamide | 12 (32.4) |
| Use of bosentan | 8 (21.6) |
| Use of calcium channel blocker | 15 (40.5) |

*Seven patients didi not undergo the examination, sample of 30 patients with Echocardiogram

age of onset of the disease varies depending on the region of the world evaluated[18]. In this sample, the population showed a later onset, at 52.21 years, than that found in studies in Brasil (50.5 years[19,20]) and Latin America, which has studies showing disease onset with an average age of 35.8 years[21].

The high prevalence of abnormal manometry findings in the present study is in agreement with the literature[3,4]. The classic dysmotilities of SS in EM are hypomotility with waves not conducted on the esophageal body concomitant with hypotonia of the lower esophageal sphincter, which occurred in 35.15% of patients in this series. However, 37.8% of the patients had only involvement of the esophageal body, without the involvement of the EIR. And 10.8% had LES abnormalities without the involvement of the esophageal body. The higher prevalence of the involvement of the esophageal body in relation to the abnormalities of the EIR, in isolation, were already known[8,22]. There are reports that SS starts in the esophagus body[23]. It is believed that arteriole changes of the *vasa nervorum* would lead successively to the three stages of esophageal involvement: neuropathy, myopathy, and fibrosis, which may explain the higher frequency of impairment of the EB musculature than that of the LES, corroborated by the findings of the present study[23]. But there is no way to tell how much time of disease progression is required to present the concomitant involvement of the esophageal body and of the LES, or if all individuals evolve in the same way. It is important to emphasize that the patients of the group studied had a mean time of 5.2 years since the first symptom until the diagnosis of SS.

Both diabetes mellitus[23] and hypothyroidism with myxedema[23] can cause esophageal abnormalities detectable by EM. It is not possible to determine in this study, due to the small number of cases with overlapping, if indeed these entities have contributed to precipitate, worsen, or not the abnormalities in the EM. In the same way, the role of calcium channel blockers on esophageal motility is controversial. That influence is controversial, but this study corroborates the findings of a previous one[22], which also found no association between the use of calcium channel blockers and the manometry findings of esophageal dysmotility or hypotonia of the LES.

In the series analyzed, there was one case of involvement of the upper sphincter of the esophagus, an unusual fact in SS[24], which was associated with the concomitant presence of inflammatory myopathy since it is formed by striated skeletal muscles[24].

In this group, there were two EM with achalasia, not usual among the classic findings of SS[22]. There is probably more than one etiopathogenic mechanism that causes damage in the esophagus in SS. The immune-mediated condition of SS is among the causes of esophageal structural damage[11]. Chagas disease was excluded in these cases[25].

No statistically significant relationship was found between esophageal body abnormalities and of the LES alone with limited and/or diffuse cutaneous subgroup. There is also no relationship between the involvement of the body and the LES with the diffuse or limited cutaneous presentation of SS. This fact is in agreement with the study developed by Calderaro at al[22], whose patients and geographical location are similar to those of this study. Unlike what was suggested by Leroy, in 1988[9], who correlated the limited cutaneous presentation with esophageal abnormalities.

There is a concern regarding the consequences of gastric content aspiration on the pulmonary parenchyma, leading to ILD. Pulmonary damage is present in 57-86% of patients with SS[2]. Studies have suggested an association of severe esophageal disease in patients with the presence of anti-topoisomerase1[10]. This, in turn, would be associated with a greater prevalence of ILD[10]. In this study, we recorded 37.8% of cases of ILD and 24.3% of cases of anti-topoisomerase 1. The high prevalence of esophageal abnormalities did not correlate with a higher frequency of abnormalities on computed tomography scans in this study. Even when analyzed separately, abnormalities exclusive to the esophageal body, or in association with LES abnormalities, showed no statistically significant association with ILD. That is perhaps because the frequency of ILD in our population was lower than that described in the literature, which might have influenced in the absence of this association.

In patients analyzed, the presence of SEI was not statistically correlated with none of the manometry abnormalities analyzed. The absence of correlation between the presence of esophageal abnormalities in EM and SEI is well described in the literature since ES is less frequent than EM abnormalities. Perhaps this can be explained by the slow progression of the disease, or by the lack of more sensitive instruments for the clinical investigation of the patient.

This study was conducted at a referral center in the countryside of Brasil and presented as a limitation a small number of individuals with SS. Thus, the data may not be extrapolated, despite corroborating

Brazilian studies conducted previously. There is also a limitation regarding the technique of EM, which was performed with conventional equipment (not high resolution), but that is the equipment available in most national services. However, this study stands out for demonstrating that there is no correlation of clinical data such as cutaneous involvement and/or SEI that denote the profile of EM abnormalities. In addition, segmental abnormalities in the esophagus in EM do not correlate with ILD. These facts require special attention from the medical team because these patients may have relevant esophageal and lung structural changes without necessarily presenting a classic clinical pattern of the disease. There is no need to wait for diffuse cutaneous SS to investigate ILD, nor is it necessary for the esophagus body to be affected concomitantly with the LES for ILD screening to be conducted. It is also not necessary to wait for SEI to investigate the organ.

## CONCLUSION

In the population with SS evaluated in this study, esophageal abnormalities in EM were frequent. However, no distribution pattern was found regarding segmental alterations, isolated or concomitant, of the esophagus with the type of cutaneous involvement. It was also not possible to establish a relationship of a specific manometry pattern and the presence of ILD. The existence of clinical gastrointestinal symptoms did not correlate with the diffuse or limited cutaneous pattern, or with manometry alterations of body and LES, isolated or associated, even when ILD was present. This study is relevant because it indicates the need for special attention in the care of patients with SS since they need to be screened for esophageal complications without SEI. It is also necessary to assess the existence of ILD, regardless of the type of cutaneous involvement and the intensity of the esophageal involvement.

### Contribution of the authors

Juliana Markus - main author, responsible for the text; Rogério de Melo Costa Pinto - Supported the statistical analysis of the data and supervised the writing.

Abadia Gilda Buso Matoso - Supported the research and the writing of the draft; Roberto Ranza - Designed the methodology and supported the review of the article.


## RESUMO

INTRODUÇÃO: *A esclerose sistêmica (ES) é uma doença autoimune que afeta vários órgãos de etiologia desconhecida, caracterizada por dano vascular e fibrose da pele e órgãos. Entre os órgãos envolvidos estão o esôfago e o pulmão.*

OBJETIVOS: *Relacionar o perfil das alterações na eletromanometria (ME), o perfil de acometimento da pele, a pneumopatia intersticial (PI) e os sintomas esofágicos em pacientes com ES.*

MÉTODO: *Trata-se de um estudo observacional, transversal, realizado no ambulatório de SSC do Hospital das Clínicas da Universidade Federal de Uberlândia. Após aprovação pelo Comitê de Ética e assinatura dos termos de consentimento, 50 pacientes foram inicialmente convidados, de 04/12/2014 a 25/06/2015. Eles foram submetidos às investigações usuais de acordo com o quadro clínico. A análise estatística foi descritiva em porcentagem, média e desvio padrão. O teste Qui-quadrado foi utilizado para avaliar a relação entre ME, tomografia de alta resolução e sintomas esofágicos.*

RESULTADOS: *91,9% dos pacientes apresentaram alterações manométricas. 37,8% tinham envolvimento do corpo esofágico e do esfíncter esofágico inferior. 37,8% tinham IP. 24,3% apresentaram a forma difusa da ES. Não há associação entre alterações manométricas e manifestações clínicas (sintomas cutâneos, pulmonares e gastrointestinais).*

CONCLUSÃO: *O presente estudo confirma que as alterações da motilidade esofágica detectadas pela EM são frequentes em pacientes com SSC, mas podem não estar relacionadas ao envolvimento cutâneo, à de DPI ou às queixas gastrointestinais dos pacientes.*


PALAVRAS-CHAVE: *Escleroderma sistêmico, Esôfago, Doenças pulmonares intersticiais, Manometria*

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ORIGINAL ARTICLE

# Evaluation of adult celiac disease from a tertiary reference center: a retrospective analysis

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## SUMMARY

OBJECTIVE: *It has been observed that celiac disease (CD) is not restricted to a single type characterized by diarrhea but also has atypical, asymptomatic (silent), and latent forms. The prevalence of this autoimmune disease, which affects approximately 1% of the world, is estimated to be around 3%, including atypical and asymptomatic cases. In our study, we aimed to evaluate adult celiac patients.*

METHODS: *Between December 2008-2015, patients diagnosed with CD over the age of 18 years old were included in the study. Patients' symptoms at admission, frequency and type of anemia, transaminase levels, and celiac antibody positivity, and autoimmune diseases diagnosed at follow up were evaluated retrospectively.*

RESULTS: *Of 195 patients, 151 (77.4%) were female. The mean age of the patients was 35.73 ± 12.19 years (range, 18-71 years). A hundred patients (51.3%) had gastrointestinal symptoms. At the time of admission, 118 patients (60.5%) had anemia, and 52 (26.7%) had hypertransaminasemia. During the mean follow-up period of 58 months (36-120 months), 84 (43.1%) of the patients presented at least one autoimmune disease, and this rate was 96.6% in individuals diagnosed above the age of 50 years.*

CONCLUSION: *In adult CD, resistant anemia, dyspepsia, and hypertransaminasemia are very common findings at the time of diagnosis, and the association with other autoimmune diseases, especially Hashimoto's thyroiditis, is high.*

KEYWORDS: *Celiac Disease. Adult. Autoimmune diseases. Anemia.*

## INTRODUCTION

Celiac disease (CD) is an immune-mediated persistent gluten intolerance that develops against gluten proteins such as barley, wheat, and rye in genetically predisposed individuals. Gluten proteins induce T-cell-associated inflammation in the small intestine and cause an autoimmune response to their own proteins, such as tissue transglutaminase.[1] This causes villous atrophy, crypt hypertrophy, and intraepithelial lymphocytosis. With the developing technology and laboratory methods, it was observed that CD is not restricted to a single form characterized by diarrhea; atypical, asymptomatic (silent), and latent forms of the disease have also been discovered. In recent studies, it was observed that its incidence and prevalence have increased in both sexes and all age groups and that it is an autoimmune disease that affects approximately 1% of the world population.[2] Since this ratio shows a biopsy-diagnosed population, it is suggested that the prevalence can be around 3% when atypical and asymptomatic cases are included.[3]

Atypical and asymptomatic cases are frequently seen in patients diagnosed in adulthood. In the

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classical form seen in adults, only half of the cases have diarrhea, bloating, and abdominal discomfort. Diarrhea is sudden and is often chronic. There are no gastrointestinal symptoms in the atypical form. Hematological, psychiatric, endocrine, renal, neurological, rheumatologic, dermatological, and cardiovascular symptoms are common in the atypical form.[4] However, most of these patients show severe mucosal damage and have a celiac-specific antibody pattern.

In our study, we aimed to perform a retrospective evaluation to determine their reason for admission, the prevalence of anemia, hypertransaminasemia at the time of diagnosis, and the frequency of autoimmune disease diagnosed in follow up or at the diagnosis in patients who were diagnosed with CD at our center over the age of 18 and were followed up for at least 3 years.

## MATERIAL AND METHODS

### Study group

Patients diagnosed with CD, with celiac antibodies positivity and duodenal biopsy between December 2008 and December 2015 were included in the study. CD was confirmed by the presence of partial, subtotal or total villous atrophy on duodenal biopsies, associated with increased intraepithelial lymphocyte counts and crypt hyperplasia, as defined by the modified Marsh criteria.[3]

### Inclusion Criteria

Patients who had transaminase levels, complete blood count (CBC), anemia panel (iron, total iron-binding capacity, ferritin, B12, folic acid), thyroid function tests (free triiodothyronine (fT3), free thyroxine (fT4), thyroid-stimulating hormone (TSH), Anti-M antibodies, Anti-T antibodies) and celiac antibodies (anti-tissue transglutaminase immunoglobulin Ig A, anti-gliadin antibody IgA and IgG, anti-endomysial Ig A and G) at the time of diagnosis were included the study.

### Exclusion Criteria

Patients diagnosed at under 18 years old, patients who had missing laboratory data and celiac antibodies at admission, patients whose duodenum and bulbous biopsies did not support CD were not included in the study.

The study was approved by the local ethical committee of the Tepecik Education and Research Hospital (No: 2019-0014).

### Evaluation of patients in the study group

Symptoms of patients at admission, frequency, and type of anemia, transaminase levels, positivity, and levels of celiac antibodies and autoimmune diseases diagnosed during follow up were evaluated retrospectively.

### Statistical analysis

Statistical analysis of the study was performed using SPSS 22.0 (IBM Statistical Package for Social Sciences software version 22). Continuous variables were expressed as mean ± standard deviation (SD) or median (minimum-maximum) and categorical variables as percentages. The Chi-square test was used to compare categorical values between groups, and the Mann-Whitney U test was used to compare continuous variables between groups. $P < 0.05$ was considered statistically significant.

## RESULTS

### Demographic characteristics of patients and distribution by age groups

A total of 195 patients (151 females, 44 males) were included in the study. The mean age of the patients was 35.73 ± 12.19 years (range, 18-71 years). A hundred of these patients (51.3%) were referred to the gastroenterology outpatient clinic because of gastrointestinal (GIS) symptoms, 90 (46.2%) for extra-GIS symptoms, and 5 (2.6%) due to family history. The distribution of patients according to age groups and their reasons for referral to the gastroenterology outpatient clinic are summarized in Table 1. The distribution of the groups at admission are summarized in Figure 1a and b.

### Serology positivity at admission

At the time of diagnosis, 185 (94.8%) of the cases had anti-tissue transglutaminase IgA, 180 (92.4%) had the anti-endomysium antibody, and 176 (90.2%) had anti-gliadin antibody positivity.

### Rates of anemia and hypertransaminasemia in patients at admission

A hundred and eighteen patients (60.5%) had anemia (hemoglobin level; female: <12, male: <13 g/L) at admission. A total of 53.3% of patients had iron deficiency anemia (IDA) (normal values of ferritin : 10-120 ng/mL, normal values of transferrin saturation: 20-40 ug/dL), 38.4% had folic acid deficiency (normal values of folic acid: 4.6-18.7 pg/mL), 25% had B12 deficiency (normal values of B12: 191-663 pg/mL) and 10.2% had

chronic disease anemia. Fifty-two patients (26.7%) had hypertransaminasemia (AST or ALT level > 35 U/L) at admission. The rates of anemia and hypertransaminasemia at the time of admission according to age groups are summarized in Figure 2a and b. In the subgroup analysis, no statistically significant difference was found between the groups in terms of anemia and hypertransaminasemia when the patients were classified as < 40 and ≥ 40 years old (Table 2).

### Rates of autoimmune diseases diagnosed during follow-up

The mean follow-up period of 195 patients with CD diagnosed between December 2008 and December 2015 was 58 months (36-120 months). During the follow-up period, it was found that 84 patients (43.1%) presented at least one autoimmune disease, and the most common was autoimmune thyroid disease (25.6%) (Table 3). The total number of autoimmune diseases was 94. The distribution of autoimmune thyroid diseases and other autoimmune diseases according to age groups is summarized in Figure 3a and b. In the subgroup analysis, it was found that there was a statistically significant difference between the groups in terms of autoimmune thyroid diseases and other autoimmune diseases when the patients were classified as < 40 and ≥ 40 years old (Table 2).

## DISCUSSION

As with other autoimmune diseases, CD is 2 to 3 times more common in females, affecting approximately 1% of the community.[5] In our study, most patients were female (female/male; 2.9), similar to the literature. The prevalence of CD diagnosed with biopsy is reported between 0.4-0.9% in our country.[6,7] Developing diagnostic methods showed that the disease indeed resembles an iceberg and can be seen in individuals with atypical symptoms and in asymptomatic individuals, as well as in those with typical symptoms. In studies, especially in an adult group, it is found that the delays in diagnosis are quite frequent because of the frequency of atypical symptoms.[4,8,9] In the study of Volta et al.[10], 86% of adult patients diagnosed in the last 5 years were found to be with non-classical and subclinical phenotypes. In the literature, we see that atypical and silent forms are more prominent than the classical symptoms in the cases diagnosed in adulthood in the last decades.[4,10] In our study, we found that almost half of the patients presented with extra-GIS symptoms. In addition, one-fifth of the cases referred for GIS symptoms were individuals with dyspeptic symptoms. In the subgroup analysis, more than 55% of patients, especially in the age groups of 30-50 and over 60, were referred with extra-GIS symptoms. The majority of these cases consisted of patients with resistant anemia and hypertransaminasemia. This shows that screening tests, such as anti-tissue transglutaminase Ig A, are important in individuals with resistant anemia, dyspepsia, and hypertransaminasemia, especially in the adult age group without typical GIS symptoms.

**TABLE 1.** DISTRIBUTION OF PATIENTS ACCORDING TO AGE AND THEIR REASONS FOR REFERRAL.

| Diagnosis age | n (%) | Referral with GIS symptoms, % |
|---|---|---|
| 18-29 y | 65 (33.3%) | 63% |
| 30-39 y | 64 (32.8%) | 40% |
| 40-49 y | 36 (18.5%) | 44% |
| 50-59 y | 21 (10,8%) | 61% |
| ≥ 60 y | 9 (4.6%) | 42% |

**TABLE 2.** THE RATES OF ANEMIA AND HYPERTRANSAMINASEMIA AT DIAGNOSIS AND THYROID AND AUTOIMMUNE DISEASES IN FOLLOW-UP AT PATIENTS < 40 AND ≥ 40 YEARS OLD.

| | < 40 y (%) | ≥ 40 y (%) | p |
|---|---|---|---|
| Anemia | 62% | 57.6% | 0.327 |
| Hypertransaminasemia | 26.4% | 27.3% | 0.510 |
| Thyroid disease | 17.8% | 40.9% | 0.001 |
| Autoimmune disease | 28.7% | 71.2% | 0.001 |

**TABLE 3.** AUTOIMMUNE DISEASES AND RATES IN CELIAC PATIENTS.

| Autoimmune disease | n (%) |
|---|---|
| Hashimato's thyroiditis | 41 (21%) |
| Graves' disease | 9 (4.6%) |
| Sjogren | 8 (4.1%) |
| Primary biliary cirrhosis | 7 (3.5%) |
| Diabetes 1 | 5 (2.5%) |
| Seropositive rheumatoid arthritis | 5 (2.5%) |
| Dermatitis herpetiformis | 5 (2.5%) |
| Systemic lupus erythematosus | 4 (2%) |
| Ulcerative colitis | 3 (1.5%) |
| Autoimmune hepatitis | 3 (1.5%) |
| Polymyalgia rheumatica | 1 (0.5%) |
| Psoriasis vulgaris | 1 (0.5%) |
| Systemic sclerosis | 1 (0.5%) |
| Ankylosing spondylitis | 1 (0.5%) |

CD is also highly associated with other autoimmune diseases.[8,11-14] In the large patient cohort study by Grode et al.[8], the disease was associated with 31 different autoimmune comorbid diseases. Studies have shown that CD is more common in patients with type 1 Diabetes Mellitus, thyroiditis, and psoriasis because of shared immune-associated pathogenesis.[11,14] The association of endocrine and dermatological events is more common in the diagnosis of CD in adulthood. The most common concomitant autoimmune disease was Hashimato's thyroiditis.[15] In the literature, the association of thyroid diseases with CD was reported as 2-24.3%.[4,11,12] The reason for this prevalence appears to be multi genetic loci such as HLA-DR3, HLA-DQ2,

**FIGURE 1.** SYMPTOM DISTRIBUTION OF PATIENTS DIAGNOSED WITH A) EXTRA-GIS SYMPTOMS B) GIS SYMPTOMS.

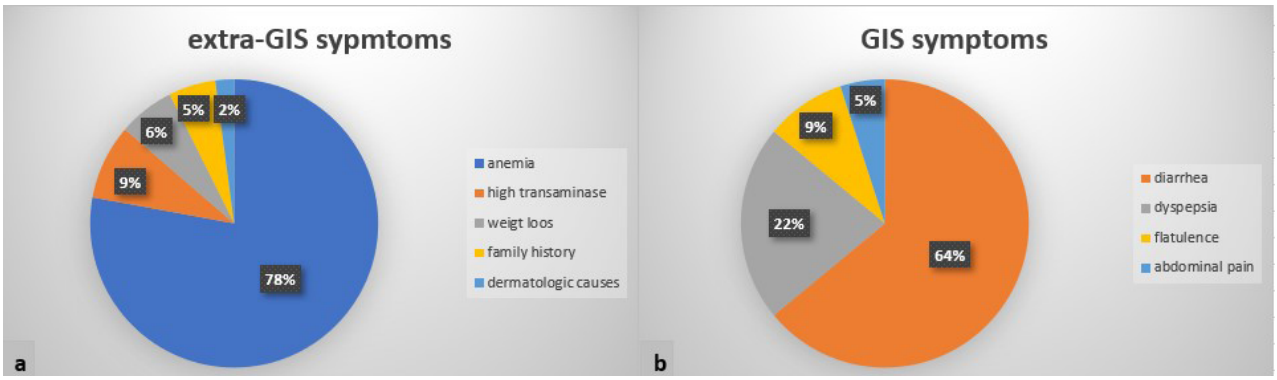



**FIGURE 2.** A) DISTRIBUTION OF ANEMIA RATES ACCORDING TO AGE GROUPS B) DISTRIBUTION OF HYPERTRANSAMINASEMIA ACCORDING TO AGE GROUPS.

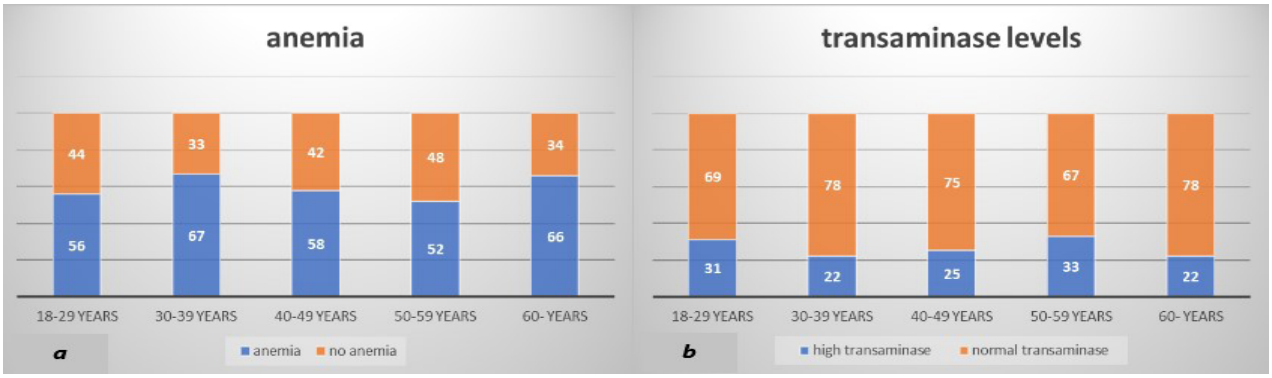
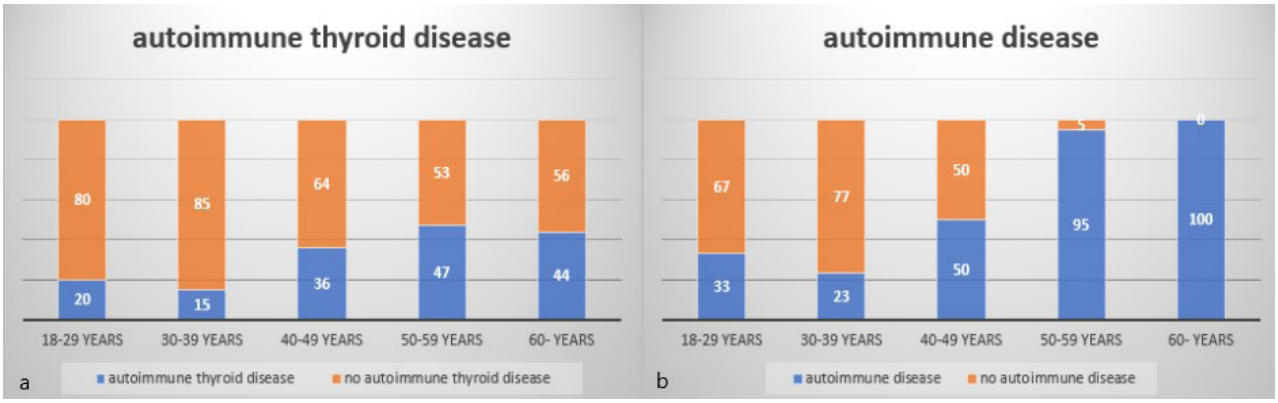



**FIGURE 3.** A) DISTRIBUTION OF PATIENTS WITH AUTOIMMUNE THYROID DISEASES ACCORDING TO AGE GROUPS DURING FOLLOW-UP B) DISTRIBUTION OF PATIENTS DIAGNOSED WITH AUTOIMMUNE DISEASE ACCORDING TO AGE GROUPS DURING FOLLOW-UP.



common to autoimmune diseases.[16] In our study, autoimmune diseases were observed in 43.1% of the cases, and autoimmune thyroid diseases were the most common with 25.6%, due to the mean follow-up period of 58 months after diagnosis. In the subgroup analysis, it was determined that there was a statistically significant difference in autoimmune disease (28.7% versus 71.2%; $p < 0.001$) and autoimmune thyroid diseases (17.8% versus 40.9%; $p < 0.001$) in individuals between < 40 and ≥ 40 years old.

In addition, it was found that anemia is quite a common finding, especially in patients with CD. In studies, the prevalence of anemia is 12-85% in patients with CD.[4,17,18] In particular, IDA is quite common due to the pathophysiology of the disease. In one meta-analysis, one of 31 patients with IDA was reported to have CD.[19] IDA was observed in 26%, folate deficiency in 12%, B12 deficiency in 5% of 405 patients according to Harper et al.'s[18] study. IDA and folic acid deficiency associated primarily with the effect of CD on the proximal small bowel mucosa are common findings, and B12 deficiency absorbed from terminal ileum is also another cause. Several hypotheses have tried to explain B12 deficiency in CD. Although it cannot be fully elucidated, one of the probabilities is the association with autoimmune gastric atrophy, which is another autoimmune disease. This may also be due to the disruption of B12 and salivary R protein (haptocorrin) complexes due to concomitant pancreatic insufficiency and associated disruption of pancreatic proteases.[20] Another possibility is that villous involvement in celiac disease is not limited to the proximal small bowel but also exists in the ileum.[21] In studies, B12 deficiency was reported in 11-41% of CD.[4,21] In our study, we found that more than half of the patients were anemic (60.1%) in all age groups at the time of admission. In accordance with the literature, it was observed that IDA (53.3%) and folic acid deficiency (38.4%) were the most frequent, and B12 deficiency (25.6%) was found in one-fourth of the cases. In the subgroup analysis, the rates of anemia were found to be similar in individuals < 40 and ≥ 40 years old (62% versus 57.6%; $p = 0.327$).

Hypertransaminasemia is a very common finding in treatment-naive patients and is seen in approximately 40% of adult cases and 60% of pediatric cases at the time of diagnosis.[22] In addition, approximately 9% of individuals with chronic unexplained hypertransaminasemia can have clinical or serological evidence of CD.[23] Studies have shown that the risk of late-stage liver disease is 2 to 6 times greater, and the risk of death due to liver cirrhosis is 8 times higher than in the normal population.[24] However, the relationship between the disease and liver damage cannot be clearly identified; in individuals susceptible, following gluten exposure to the abnormal intestinal permeability due to a predisposition of common genetic factors, such as HLA associated with autoimmunity, cytokines, autoantibodies, and various biological mediators are thought to play a role in the pathogenesis.[25] In our study, close to one-fourth of the cases (26.7%) had hypertransaminasemia at the admission. In addition, 2 patients (1%) diagnosed with cirrhosis were also diagnosed with CD based on the results of celiac antibodies and duodenal biopsy, although they were asymptomatic. In the subgroup analysis, hypertransaminasemia was found to be similar in individuals < 40 and ≥ 40 years old (26.4% versus 27.3%; $p = 0.51$).

Since our study is a retrospective study, it has some limitations. First, there is a lack of data for many patients regarding the duration of symptoms in the past during their referral to the outpatient clinic. This prevents us from having data about the delay of the diagnosis. Secondly, due to the lack of detailed data on the other causes of anemia, such as diet, menstrual cycle-related excessive bleeding, anemia rates reflect the overall rates of anemia seen in CD at the time of diagnosis. Thirdly, it is useful to note that the cases with hypertransaminasemia at the time of diagnosis had not only CD but should also be associated with other diseases that cause hypertransaminasemia, such as hypothyroidism, as well as concomitant autoimmune liver disease.

## CONCLUSIONS

In conclusion, CD patients may not present typical symptoms and signs in adulthood. Especially in individuals with unexplained resistant anemia, dyspepsia, and hypertransaminasemia, screening tests for CD are important. The disease is frequently associated with other autoimmune diseases due to its autoimmune character. We think that, especially in cases diagnosed above the age of 40 years, it is necessary to conduct an examination and screening tests, especially for a second comorbid such as endocrine and rheumatic autoimmune diseases.

### Conflict of interest

None.

## Authors' Contribution

Concept: OBB. Design: OBB. Supervision: FT. Materials: OBB, FT. Data collection and processing: FT. Analysis and interpretation: OBB, FT. Literature search: OBB. Writing: OBB. Critical reviews: OBB.


### RESUMO

OBJETIVOS: *Observou-se que a doença celíaca (DC) não se restringe a um único tipo caracterizado por diarreia, mas também tem formas atípicas, assintomáticas (silenciosas) e latentes. Estima-se que a prevalência desta doença autoimune, que afeta aproximadamente 1% da população do mundo, seja em torno de 3%, incluindo casos atípicos e assintomáticos. Em nosso estudo, objetivou-se avaliar pacientes celíacos adultos.*

MÉTODOS: *Entre dezembro de 2008 e 2015, pacientes diagnosticados como DC com idade acima de 18 anos foram incluídos no estudo. Os sintomas dos pacientes na admissão, frequência e tipo de anemia, níveis de transaminases e positividade de anticorpos celíacos e doenças autoimunes diagnosticadas no seguimento foram avaliados retrospectivamente.*

RESULTADOS: *Dos 195 pacientes, 151 (77,4%) eram do sexo feminino. A média de idade dos pacientes foi de 35,73±12,19 anos (variação de 18 a 71 anos). Cem pacientes (51,3%) foram encaminhados com sintomas gastrointestinais. No momento da internação, 118 pacientes (60,5%) apresentavam anemia e 52 (26,7%) apresentavam hipertransaminemia. Durante o período médio de acompanhamento de 58 meses (36-120 meses), 84 (43,1%) pacientes estavam acompanhados por pelo menos uma doença autoimune, e essa taxa foi de 96,6% em indivíduos diagnosticados acima dos 50 anos de idade.*

CONCLUSÃO: *No adulto DC, anemia resistente, dispepsia e hipertransaminasemia são achados muito comuns no momento do diagnóstico e a associação com outras doenças autoimunes, especialmente tireoidite de Hashimoto, é alta.*


PALAVRAS-CHAVE: *Doença celíaca. Adulto. Doenças autoimunes. Anemia.*

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ORIGINAL ARTICLE

# Laboratory predictors of survival in ovarian cancer

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## SUMMARY

OBJECTIVE: *To relate disease-free survival and overall survival with type I and type II ovarian cancer and preoperative laboratory parameters biomarkers.*

METHODS: *A retrospective study was carried out based on the collection of data from medical records of patients with ovarian tumors. Kaplan-Mayer curves were drawn based on the statistical analysis of the data and were compared using the Log-rank test.*

RESULTS: *Disease-free survival in type I ovarian cancer was significantly higher than in type II (p=0.0013), as well as in those with normal levels of CA-125 (p=0.0243) and with a platelet-lymphocyte ratio (PLR) lower than 200 (p=0.0038). The overall survival of patients with type I ovarian cancer was significantly higher than in patients with type II, as well as in patients with normal CA-125 serum levels (p=0.0039) and those with a preoperative fasting glucose of less than 100 mg/dL.*

CONCLUSION: *CA-125 levels may predict greater overall and disease-free survival. PLR < 200 may suggest greater disease-free survival, whereas normal fasting glucose may suggest greater overall survival.*

KEYWORDS: *survival, ovarian neoplasms, glucose, CA-125 Antigen.*

## INTRODUCTION

Ovarian cancer is the fifth leading cause of cancer mortality in women[1]. According to data from Globocan[2], an estimated total of 238,719 new cases were diagnosed worldwide in 2012. Many studies have evaluated the clinical relevance of potential biomarkers, such as tissue or serum samples from patients with ovarian cancer, for their ability to predict either chemotherapy response or survival[3].

Both basic and translational research has shown that ovarian cancer includes several types of tumors with different phenotypes, molecular biology, etiology, progression, and even prognosis. In 2014, Shih & Kurman[4] proposed a classification system for ovarian tumorigenesis based on morphology and genetic molecular analysis. In this model, ovarian epithelial tumors are divided into two broad categories, designated as type I and type II. Type I tumors tend to be low grade, slow-growing neoplasms, and are associated with distinct molecular changes. They are relatively genetically stable and rarely have P53

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mutations. There have already been many studies of the mutations that typically occur in type I tumors, such as the BRAF and KRAS mutations for serous tumors, KRAS mutations for mucinous tumors, and β-catenin and PTEN mutations for endometrioid tumors. On the other hand, type II tumors are high-grade neoplasms with accelerated and disorganized growth, and very often, their precursor lesions have not been morphologically identified, so they tend to be diagnosed in more advanced stages. They are genetically unstable and have a high frequency of P53 mutations[5]; however, beyond that, the data on their molecular changes are still very limited. Thus, patients with type II tumors present a much higher chance of recurrent disease when compared to patients with type I tumors[6].

There is an important need for the development of new biomarkers for the diagnosis and prognosis of ovarian cancer, and ideally, these biomarkers would also serve as targets for new therapeutic modalities[7]. Systemic inflammatory response markers, such as absolute white blood cell count, neutrophil-lymphocyte ratio (NLR), and platelet- lymphocyte ratio (PLR), have been used as prognostic factors in patients with various types of tumors[8,9]. It is proposed that they can be used as significant predictors of malignancy for solid tumors originating from various tissues, also revealing that they can be used as a screening tool for these tumors as they are considered low cost and readily available tests. However, it is necessary to further research to evaluate the additional value of this finding to establish scores and indicate the potential predictive value of these markers in gynecological cancers[10].

Our study aims to correlate disease-free survival (DFS) and overall survival (OS) with type 1 and type 2 ovarian cancer and with preoperative laboratory parameters.

## PATIENTS AND METHODS

This retrospective study was carried out using the medical records of patients with ovarian tumors being treated at the Pelvic Mass Ambulatory and undergoing surgical treatment by exploratory laparotomy according to pre-established criteria[11,12], and subsequently diagnosed with malignant ovarian neoplasia.

The inclusion criterion was a postoperative diagnosis of primary malignant ovarian neoplasia (epithelial or non-epithelial tumors) by anatomopathological paraffin analysis. Exclusion criteria were secondary malignant ovarian neoplasia (metastasis); torsion of the adnexal pedicle; treatment prior to surgery; recurrence; diseases that cause immunosuppression; treatment with immunosuppressive drugs.

The study was approved by the Research Ethics Committee (protocol number 2,061) and performed in accordance with the ethical standards as laid down in the 2013 Declaration of Helsinki. Informed consent was obtained from all participants.

### Patients

Patients with confirmed histological diagnosis of ovarian cancer had the following data recorded: age, histological type, histological grade, staging (FIGO), type I/II classification (for epithelial tumors), lymph node metastases, OS, and DFS. Hemoglobin, absolute neutrophil and lymphocyte values, platelets, fasting glucose, and preoperative tumor markers (CA125, CA15.3, CA19.9) were also obtained from laboratory tests.

The NLR and PLR values were obtained by dividing the absolute number of neutrophils and platelets, respectively, by the absolute number of lymphocytes. The cut-off values used for NLR and PLR were 4 and 200, respectively[13,14].

DFS was considered from the date of histopathological diagnosis of ovarian cancer to the date of the first relapse. OS was calculated from the date of histopathological diagnosis of ovarian malignancy to death from any cause.

### Statistical analysis

Data were analyzed in GraphPad Prism software 7. DFS and OS were assessed using Kaplan-Meier curves and compared using the log-rank test, with significance set at $p<0.05$. Considering the estimated proportion of death and relapse in the sample, at least 88 patients would be required to obtain a test power of 95%, at a significance level lower than 0.05 (www.lee.dante.br).

## RESULTS

In total, the medical records of 110 patients diagnosed with malignant ovarian neoplasia were analyzed. The median age was 51 years (12-82). Results of preoperative laboratory tests are shown in Table I.

Serous cystadenocarcinomas represented the most common histological type, found in 30 (27.3%)

patients. There were 20 (18.9%) granulosa cell tumors, 16 (14.5%) borderline mucinous tumors, 10 (9.1%) borderline serous tumors, 6 (5.5%) mucinous cystadenocarcinomas, 4 (3.6%) endometrioid tumors, 4 (3.6%) adenocarcinomas, 4 (3.6%) dysgerminomas, 3 (2.7%) clear cell tumors, 2 (1.8%) carcinosarcomas, 2 (1.8%) endodermal sinus, 2 (1.8%) teratoma immature, 1 (0.9%) embryonal carcinoma, 1 (0.9%) borderline endometrioid, 1 (0.9%) undifferentiated stroma tumor, 1 (0.9%) poorly differentiated neoplasia, 1 (0.9%) Sertoli, 1 (0.9%) mucinous and serous, 1 (0.9%) clear cells + granulosa.

Regarding the type of carcinogenesis, 48 (43.6%) patients had ovarian cancer type I, 30 (27.2%) had ovarian cancer type II, and 32 (29.1%) were not classified because they were not epithelial cells. Regarding staging, 59 (53.6%) were in stage I, 5 (4.5%) in stage II, 36 (32.7%) in stage III, and 10 (9.1%) in stage IV. Thirty-three percent of the patients died.

DFS was significantly higher in type I than in type II ovarian cancer (p = 0.0013, Figure 1A) and was also higher in patients with levels of CA-125 lower than 35 U/ml (p = 0.0243, Figure 1B) and PLR lower than 200 (p = 0.0038, Figure 1C). There was no significant difference in DFS in relation to fasting glucose, hemoglobin, NLR, or serum levels of CA 19.9 and CA 15.3.

The OS of patients with type I ovarian cancer was significantly higher than that of patients with type 2 (p<0.0001, Figure 1D). In addition, OS was higher in patients with CA-125 serum levels lower than 35 U/ml (p = 0.0039, Figure 1E) and with preoperative fasting glucose lower than 100 mg/dL (p=0.0393, Figure 1F). There was no statistical significance regarding hemoglobin, serum levels of CA 19.9 and CA 15.3, NLR, or PLR.

## DISCUSSION

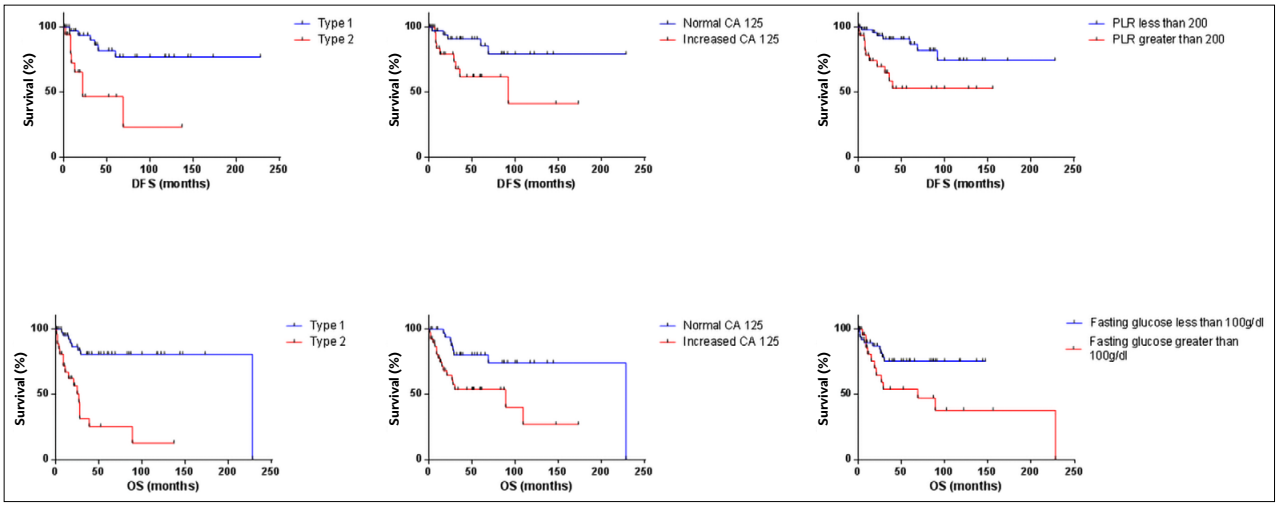
The identification of diagnostic markers for ovarian cancer, determination of prognosis, and treatment orientation is important. The most commonly used tumor marker in ovarian cancer is Serum Cancer Antigen 125 (CA-125). It was first identified by Bast, Knapp et al. in 1981[15]. CA125 is a high molecular weight glycoprotein expressed by 80% of ovarian cancers of epithelial origin and may be used to distinguish malignant pelvic masses from benign, monitor therapeutic response, and detect recurrent diseases[16,17].

**TABLE 1.** LABORATORIAL PARAMETERS.

| | Median |
|---|---|
| Fasting glucose (mg/dl) | 91.4 (48.0-379.1) |
| RBCs* ($10^6$uL) | 4.3 (2.4-6.3) |
| Hemoglobin (g/dL) | 12.4 (6.7-17) |
| Platelets ($10^3$uL) | 291 (24-729) |
| Neutrophils ($10^3$uL) | 5.487 (1.383-19.532) |
| Lymphocytes ($10^3$uL) | 1.723 (336-4.068) |
| NLR | 2.6 (0.5-31,66.6) |
| PLR | 170.2 (13.5-1,827.3) |
| CA125 (U/ml) | 54.4 (3.0-14,700) |
| CA-19.9 (U/ml) | 11.8 (0.7-706.1) |
| CA-15.3 (U/ml) | 22.9 (7.7-813) |

*RBCs: red blood cells

**FIGURE 1.** DISEASE-FREE SURVIVAL AND OVERALL SURVIVAL CURVES (KAPLAN-MEIER AND LOG-RANK TEST).




a) DFS in type I ovarian cancer was significantly higher than in type II (p=0.0013); b) DFS was higher in patients with normal levels of CA-125 (p=0.0243); c) DFS was higher in patients with PLR less than 200 (p=0.0038); d) OS in type I ovarian cancer was significantly higher than in type II (p< 0,0001); e) OS was higher in patients with normal CA-125 serum levels) (p=0.0039); f) OS was higher in patients with fasting glucose lower than 100 mg/dL (p=0.0393).

Studies have evaluated the prognostic significance of CA-125 levels at different treatment times to determine their correlation with prognosis, but their role remains controversial[18]. Such results may be related to a failure to consider tumor grade and histological type[19]. Ovarian cancer is not a single entity disease but comprises a heterogeneous group of tumors with different histological types, with well-differentiated clinical-pathological characteristics and biological behavior[4,20].

Chen et al. (2013) found that baseline levels of CA-125 in serum were higher in patients with type II ovarian cancers, with a worse prognosis. They also found that CA-125 alone was not able to predict whether the tumor was a type I or type II[21]. Our results agreed with theirs, as patients with type I tumors showed better prognosis with higher DFS and OS, as well as lower levels of CA-125.

Cell growth is controlled by a coordinated response between growth factors and nutrients. Increased basal glucose may be involved in the carcinogenesis of gynecological tumors by acting as an energy source. There are many studies associating diabetes with the prognosis of gynecological cancer patients, glycemic rates with tumor progression, and overall survival since the most common alteration in the cellular metabolism of neoplastic cells involves increased glucose[22].

Malignant tumors require a high demand for glucose and alter cellular metabolism to maintain their survival. Metabolic changes are necessary to sustain cell division and unrestricted growth[23]. As tumor cells progress, they change their morphology and organization, increase their growth rate, and acquire an increasingly glycolytic phenotype[24]. Among the main metabolic alterations of cancer cells is the so-called Warburg effect, which consists of increasing glycolysis under aerobic conditions and uptake of glucose through an excessive expression of its transporters. The Warburg effect is a metabolic characteristic associated with cancer cells, in which they preferentially use glycolysis for energy production rather than oxidative phosphorylation to produce lactate[25,26]. Changes in glucose metabolism have also been associated with therapeutic resistance in the treatment of ovarian cancer[24]. This is in agreement with our findings, in which OS was higher in patients with normal fasting glucose.

Laboratory quantification of systemic inflammatory response markers such as NLR and PLR has been shown to be a useful prognostic factor in patients with various types of cancer, including ovarian[4]. Several inflammatory mediators are induced by inflammatory or tumor cells and participate in the formation of cancer, acting as growth factors or angiogenic. In addition, immune function is compromised by mediators of the systemic inflammatory response, which increases leukocyte, neutrophil, platelet, C-reactive protein, and fibrinogen levels and decreases lymphocyte concentrations[26]. Thrombocytosis is identified in 20% to 50% of cases of ovarian cancer. One retrospective study demonstrated that a high number of platelets is related to lower OS in ovarian cancer[17]. In our study, DFS was higher in patients with PLR lower than 200.

A study showed that preoperative PLR was an independent prognostic factor in patients with ovarian cancer[13]. PLR is a reproducible and cheap laboratory hematology marker that is being suggested as a marker of thrombotic and inflammatory conditions[26]. Preoperative thrombocytosis was an unfavorable predictor of survival in patients with ovarian cancer[27]. Platelet activation and aggregation occur in response to the release of inflammatory cytokines, and thrombocytosis not only promotes invasion and metastasis of tumor cells but may also reflect a state of systemic inflammation[28].

Since our sample included 110 patients, the test power was greater than 95%. Nevertheless, there is still no well-defined cutoff value in the literature that relates PLR and NLR to prognosis in ovarian cancer. Thus, additional studies are needed to elucidate the role of new predictors of DFS and OS in ovarian cancer.

## CONCLUSIONS

Patients with type I ovarian cancer had greater DFS and OS (better prognosis) than patients with type II. CA-125 levels may be predictive of OS and DFS. PLR may suggest a higher DFS, and normal fasting glucose suggests a greater OS. Thus, low-cost and easy-to-use laboratory assessments could guide the oncologist to more appropriate treatment, and perhaps even point to future targets and novel approaches for treating ovarian cancer.

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## Declaration of interest

The authors report no conflicts of interest. The manuscript has been read and approved by all the authors, the requirements for authorship as stated earlier in this document have been met, and each author believes that the manuscript represents honest work.

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**RESUMO**

OBJETIVO: *Relacionar a sobrevida livre de doença e sobrevida global com câncer de ovário tipos I e II, assim como com parâmetros laboratoriais pré-operatórios biomarcadores.*

MÉTODOS: *Estudo retrospectivo realizado com base na coleta de dados de prontuários de pacientes com tumor ovariano. As curvas de Kaplan-Mayer foram realizadas em relação à análise estatística dos dados, sendo comparadas pelo teste de Log-rank.*

RESULTADOS: *A sobrevida livre de doença nas pacientes com câncer de ovário tipo I foi significativamente maior do que nas pacientes com câncer de ovário tipo II (p = 0,0013), bem como maior naquelas com níveis normais de CA-125 (p = 0,0243) e com relação plaquetas-linfócitos (RPL) inferior a 200 (p = 0,0038). A sobrevida global de pacientes com câncer de ovário tipo I foi significativamente maior do que em pacientes com tipo II, maior em pacientes com níveis séricos normais de CA-125 (p = 0,0039) e naquelas com glicemia de jejum pré-operatória menor que 100 mg / dL.*

CONCLUSÃO: *Os níveis de CA-125 podem predizer uma sobrevida global e livre de doença. A RPL < 200 pode sugerir uma maior sobrevida livre de doença, enquanto uma glicemia normal de jejum, uma maior sobrevida global.*

PALAVRAS-CHAVE: *sobrevida, neoplasia ovariana, glicemia, biomarcadores.*

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ORIGINAL ARTICLE

# Association between changes in body fat distribution, biochemical profile, time of HIV diagnosis, and antiretroviral treatment in adults living with and without virus infection

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## SUMMARY

OBJECTIVES: *Individuals living with HIV seem to be more prone to changes in the redistribution of body fat, characterized as lipodystrophy, which may occur in conjunction with metabolic diseases. In the present study, such impacts were assessed in adults with and without HIV and associated with the time of virus diagnosis and treatment with antiretroviral.*

METHODS: *A cross-sectional study with 123 adults, in which 87 had HIV and 36 without HIV, of both sexes, in outpatient follow-up at the Specialized Care Service (SAE) in Macaé-RJ. The following were made: 1) Alteration in body fat distribution, measured by anthropometric parameters and self-reported lipodystrophy; 2) Biochemical profile; 3) Association between HIV diagnosis time and antiretroviral treatment.*

RESULTS: *54.47% (n = 67) males, 45.52% (n = 56) females, mean age 37 years. Of these 87 were people living with HIV, 29% (n = 25) had self-reported lipodystrophy, mean time of virus infection, and antiretroviral treatment (5.80 ± 4.56 and 5.14 ± 3.82 years), respectively. Patients with self-reported lipodystrophy had a greater change in body fat distribution between 3-6 years of HIV diagnosis and a negative cholesterol profile. The antiretroviral treatment time influenced total cholesterol and triglycerides, even for patients without self-reported lipodystrophy, with a further nine years under treatment.*

CONCLUSION: *In this study, the negative cholesterol profile was mainly related to antiretroviral treatment time, even for patients without self-reported lipodystrophy, and changes in body fat distribution, measured by anthropometry, was especially associated with time for HIV infection in those with lipodystrophy self-reported.*

KEYWORDS: *Lipodystrophy; Profile Biochemistry; HIV.*

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## INTRODUCTION

Globally, there are 21.7 million people living with HIV (PVHIV) and undergoing antiretroviral treatment (ARVT). Recently, Unaids (2018)[1] stated that the efficient way to control the HIV/Aids epidemic must be the universal treatment of all infected individuals. However, this must be individualized, avoiding adverse effects in the long term.

The use of antiretroviral therapy has increased life expectancy by up to 37 years in HIV-infected patients, although changes in the redistribution of body fat, characterized as lipodystrophy, can lead to serious health problems, especially for individuals with an accumulation of visceral fat, which may happen with or without metabolic changes[2].

The world literature has not yet reached a consensus on the specific criteria and predictive models for the diagnosis of lipodystrophy in HIV-infected patients. The clinical signs observed can be confirmed by anthropometric measures, radiological or imaging exams, or through an evaluation of the subjective symptoms reported by patients conducted by the health team that follows them up, or a combination of these.[3]

Thus, the present study aimed to check for changes in body fat distribution among individuals living with and without HIV and establish an association between self-reported lipodystrophy (SL), duration of HIV infection (THIV), and treatment with antiretroviral therapy (TTO) in those infected by the virus.

## METHODS

This was a cross-sectional study with 123 adults of both sexes, aged between 18 and 59 years, of which 87 were infected with HIV and 36 were not, treated at the outpatient clinic of the Specialized Care Service (SAE) of the STI/AIDS and Viral Hepatitis Program, in the municipality of Macaé - RJ, Brasil. The data collection happened between July 2016 and February 2017, and the exclusion criteria were: pregnant women, children, adolescents, elderly individuals, and those with a previous diagnosis of cardiovascular disease (CVD).

All volunteers were informed about the study and signed a Free and Informed Consent Form (ICF), which was approved by the research ethics committee of UFRJ - Macaé Campus under CAAE: 55102516.0,0000.5699, under which this study is part of a larger project which is still in progress.

The criteria used to assess self-reported lipodystrophy (SL) was the patient's perception of changes regarding the redistribution of body fat after the HUV diagnosis, based on the "Smart Study"[4] and a tool used in the study by Soares[5]. We also used different anthropometric parameters to measure the distribution of body fat, which was characterized by the fat and/or muscle mass loss in the upper and/or lower limbs (lipoatrophy), the accumulation of fat in the central region of the body (lipohypertrophy), and the combination of both (mixed lipodystrophy), in accordance with Guidelines for Managing HIV in Adults[6].

For the biochemical profile, we considered: total cholesterol (TC), triglycerides (TG), HDL-cholesterol, LDL-cholesterol, according to Faludi et al.[7], fasting glycemia, per the Brazilian Society of Diabetes[8], TCD4 lymphocyte count, and viral load[6].

The Body Mass Index (BMI) was determined according to the WHO[9], the waist perimeter (WP) according to the NIH[10], as recommended by the Guidelines for HIV in Adults[6] and the waist/height ratio (WHR) based on Ashwell e Hsieh[11]. For the neck perimeter (NP) we considered the cutoff point of ≥39.5 cm for men and ≥36.5 cm for women as excess weight, per Ben-Noun And Laor[12]. The body fat (BF) was assessed in kilogram (kg), and the cutoff point for the body fat index (BFATI) had its cut-off point in percentiles between 5.5-8.3 for women and 4.0-5.7 for men, per Schutz et al.[13], and the percentage of body fat (BF%) was measured according to Durnin and Womersley[14], by the sum of skinfolds, in which the anatomical points, the technique, and classification were obtained based on the recommendations by Lohman[15]. The triceps skinfold (TSF) was measured with the aid of an adipometer of the Cescorf Innovare3® brand, Curitiba - PR, Brasil, three times; the cutoff point for eutrophy was 10.0-11.5 mm for men, and 18.5-26.0 mm for women, per Frisancho[16]. The arm perimeter (AP) for males was 30.7-32.3 cm and 26.9-30.9 cm for females[16]; the corrected arm-muscle area (AMAc) was 49.4-54.0 and 28.3-34.7 cm, respectively, for both genders[2,16]. We obtained the lean mass (LBM) in kilogram (kg), and the skeletal muscle mass (SMM) in kg was determined using the equation proposed by Lee et al.[17].

To analyze the variables based on sex, we used the Student's t-test to assess variables with normal distribution; for those that did not present normal distribution, we used the Mann-Whitney test. To compare the groups with TTO and THIV, Anova was used. In all analyses, we considered a significance level of 5% in the SPSS software, version 19.

## RESULTS

We included 123 people living with and without HIV, 54.47% (n=67) males, and 45.52% (n=56) females, with an average age of 37.5±10.58 years. Among these, 87 were infected by the virus, with an average time of HIV diagnosis (THIV) and treatment with antiretroviral therapy (TTO) of, respectively, 5.80±4.56 and 5.14±3.82 years. They had been under 20% (n=25) ARVT for over 10 years, 70% already exposed to some type of regimen using a protease inhibitor (PI), with an undetectable viral load (<50 copies/mL) in 80% (n=63), TCD4 counts (≥350 cells/mm³) at 95% (n=73) and (≤200 cells/mm³) in 15% (n=4), demonstrating good adherence to antiretroviral therapy.

In the interview, 28.73% (n=25) reported changes in body fat distribution perceived after the HIV diagnosis, characterizing reported lipodystrophy (RL); 18.39% (n=16) denied that, and 34.48% (n=30) were not able to answer and/or ignored the question. Among women, 64% (n=16) reported RL, noting especially central lipo-hypertrophy in 52% (n=13) and 40% of mixed lipodystrophy (n=10). Among men, 36% (n=9) confirmed RL, with frequency of 44.4% (n=4) of lipo-hypertrophy, 66.6% (n=6) of mixed lipodystrophy, and 77.7% (n=7) of lipoatrophy (Figure 1).

In Table 1, per BMI, we can see eutrophia for men with HIV and pre-obesity for the other participants. The BF% ranked men without HIV with obesity (p=0.001) and women, regardless of HIV, ranked above average, with a significantly higher value for those without HIV (p=0.032). Men with HIV showed a significantly greater loss of BF (p=0.001); LBM (p=0.010); SMM (p=0.001); TSF (p=0.001) and BP (p=0.022), compared to those without HIV, characterizing a loss of body fat, musculoskeletal, indicative of mixed lipodystrophy. For women with HIV, the significant TSF loss of body fat in the upper limbs (p=0.024) stood out, indicating the presence of lipoatrophy. The WHR and WP parameters indicated fat accumulation in the central region in all women, regardless of HIV. However, for men, we found significant values for WHR (p=0.008) and WP (p=0.037) in individuals without HIV, indicating that, among them, the excess weight seems to be directly correlated to higher risks due to the accumulation of fat in the abdominal region (lipohypertrophy), according to these parameters.

In Table 2, the results characterized a greater change in the behavior of body fat distribution for people infected with HIV for 3-6 years and with self-reported lipodystrophy, measured by BMI, WP, and AMAc; the cholesterol profile was negative, based

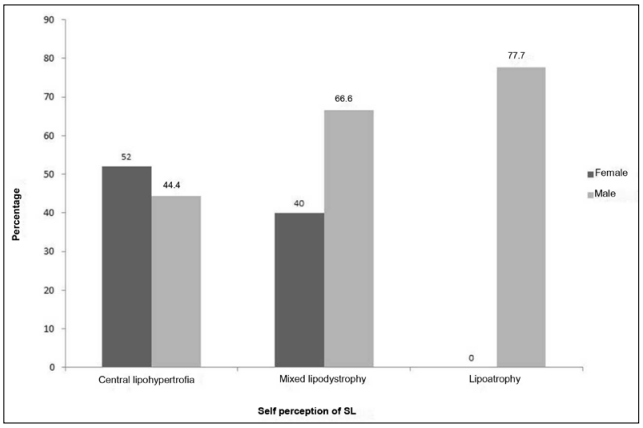


**FIGURE 1**

**TABLE 1.** CHANGES IN BODY FAT DISTRIBUTION ACCORDING TO ANTHROPOMETRIC PARAMETERS IN MEN AND WOMEN LIVING WITH AND WITHOUT HIV, ASSISTED BY THE SAE/STI/AIDS IN MACAÉ - RJ, 2018

| VARIABLES | WOMEN | | | MEN | | |
|---|---|---|---|---|---|---|
| | With HIV (Mean ± SD) | Without HIV (Mean ± SD) | P-value | With HIV (Mean ± SD) | Without HIV (Mean ± SD) | P-value |
| BMI | 27.91±7.08 | 28.50±5.35 | 0.733 | 23.94±4.26 | 29.30±6.44 | 0.001** |
| BF% | 25.66±4.12 | 27.93±3.64 | 0.032* | 23.37±5.19 | 28.65±3.92 | 0.001* |
| BF | 18.85±7.35 | 20.95±6.10 | 0.252 | 16.97±6.20 | 25.79±8.16 | <0.001* |
| LBM (kg) | 49.61±12.37 | 52.68±9.08 | 0.302 | 54.54±9.23 | 62.58±10.98 | 0.010* |
| BFATI | 7.08±2.61 | 8.10±2.30 | 0.125 | 5.68±2.02 | 8.58±2.83 | <0.001** |
| SMM (kg) | 21.21±9.38 | 22.31±3.20 | 0.595 | 28.42±4.24 | 33.30±3.89 | <0.001** |
| WHR | 0.54±0.08 | 0.55±0.07 | 0.796 | 0.48±0.09 | 0.57±0.13 | 0.008* |
| WP | 86.88±13.95 | 88.80±12.29 | 0.573 | 85.14±10.33 | 99.27±21.25 | 0.037* |
| TSF (mm) | 19.89±9.27 | 25.07±7.41 | 0.024* | 16.11±9.02 | 26.90±10.43 | 0.001* |
| AP (cm) | 30.30±5.77 | 31.67±4.99 | 0.341 | 29.35±3.89 | 32.36±4.57 | 0.022* |

Note: BMI = body mass index; BF = body fat; BF% = body fat % by skinfold addition; LBM = lean body mass; BFATI = body fat adjusted index; SMM = skeletal muscle mass; WHR = Waist/height; WP = waist perimeter; TSF = triceps skinfold; AP = arm perimeter. *T-Test ** Mann-Whitney – p<0.05.

on TC and LDC-c, which also presented higher average values for those with between 3-6 years of HIV diagnosis than between 0-3 years, characterizing that every three years of infection indicated a negative cholesterol profile for those infected who have self-reported lipodystrophy, although TC was higher among those with 6-9 years of HIV diagnosis than among those with 0-3 years. For patients without lipodystrophy, AMAc had higher values for those infected by the virus for over nine years. TC was higher among individuals with 6-9 years of HIV diagnosis. For the LDLc and TG, in individuals with more than 9 years of infection, the average values were greater than in those with 0-3 years, indicating that even for those without lipodystrophy, the time of infection negatively influenced these biochemical parameters (TC, LDL-c, and TG).

In Table 3, the results showed that for every three years of treatment regimens (TTO), there was a trend of worsening in the lipid profile (TC, LDL-c, and TG) for people living with HIV with self-reported lipodystrophy. For those classified without self-reported lipodystrophy, the parameter that stands out is the corrected arm-muscle area (AMAc), for which those with over nine years of TTO presented higher average values than those with 0-3 years of ARVT, who already show mild impairment of muscle mass in the upper limbs, according to this parameter. In the biochemical profile, the TC and TG of individuals with over nine years of treatment presented higher average values than those with 0-3 years of ARVT, indicating that, for these parameters, the time of antiretroviral use influenced the cholesterol profile, even for patients without lipodystrophy.

## DISCUSSION

In the present study, we emphasize that antiretroviral therapy promoted greater life expectancy among people infected with HIV; however, it is associated with increased body adiposity and chronicity of the inflammatory status, causing a series of endocrine-metabolic abnormalities[18.19].

There are several reports on the prevalence of lipodystrophy, ranging from 8% to 84%, with an average of 42%, particularly in regimens containing PI, due to differences in diagnostic criteria, selection of the study population, and duration of follow-up[1,2,6]. The literature indicates that patients under use PI for a minimum of 18 months were two times more likely to

**TABLE 2.** ASSOCIATION BETWEEN ANTHROPOMETRIC, BIOCHEMICAL PROFILE, THIV WITH AND WITHOUT SELF-REPORTED PRESENCE OF LIPODYSTROPHY OF PVHIV, ASSISTED BY SAE/STI/AIDS IN MACAÉ - RJ, 2018

| Time of THIV | | | | |
|---|---|---|---|---|
| With self-reported lipodystrophy | | | | |
| | 0\| - \|3 years | 3 - \|6 years | 6 - \|9 years | >9 years |
| BMI | 23.96± 3.87[a] | 34.48±13.37[b] | 23.72±2.25[a] | 25.72± 4.35[a] |
| WP | 86.78± 11.23[a] | 112.60±17.15[b] | 86.00± 7.87[a] | 94.70±11.52[a,b] |
| SMM | 23.35±4.42[a] | 24.86±2.92[a] | 25.13±6.18[a] | 22.36±5.65[a] |
| AMAc | 28.72± 10,64[a] | 58.13±8.57[b] | 46.58±22.49[a,b] | 39.09±11.26[a,b] |
| BF% | 24.28±6.25[a] | 23.47±8.38[a] | 22.74±3.00[a] | 25.73±3.68[a] |
| CT | 180.60± 72,21[a] | 268.33±54.51[b] | 201.80±58.79[a,b] | 233.00±92.37[a,b] |
| LDL-c | 108.60± 46,58[a] | 186.33±61.80[b] | 134.80±40.56[a,b] | 128.60±44.39[a,b] |
| TG | 151.60± 67,88[a] | 140.66± 14,97[a] | 129.00± 54,1[a] | 149.00± 72,44[a] |
| GLU | 109.60± 55,22[a] | 109.00± 6,00[a] | 90.60± 7,43[a] | 100.27± 26,11[a] |
| Without self-reported lipodystrophy | | | | |
| | 0\| - \|3 years | 3 - \|6 years | 6 - \|9 years | >9 years |
| BMI | 23.59±4.28[a] | 22.50±6.23[a] | 25.59±2.75[a] | 26.98±1.60[a] |
| WP | 86.25±11.89[a] | 85.66±16.25[a] | 93.37±9.79[a] | 99.30±5.24[a] |
| SMM | 29.70±4.20[a] | 20.97±9.55[a] | 22.04±2.54[a] | 22.60±5.96[a] |
| AMAc | 25.17±4.43[a] | 32.80±20.27[a] | 39.83±5.58[a] | 44.56±8.81[a] |
| BF% | 25.94±5.93[a] | 24.55±0.83[a] | 26.76±6.03[a] | 23.8±4.46[a] |
| CT | 183.00± 34.03[a] | 195.00±47.62[a,b] | 244.25±39.73[b] | 226.66±44.92[a,b] |
| LDL-c | 109.25± 27.50[a] | 137.00±50.21[a,b] | 146.25±14.31[a,b] | 158.00±50.72[b] |
| TG | 96.00± 30.88[a] | 120.66±36.69[a,b] | 181.25±227.09[a,b] | 307.00±276.79[b] |
| GLU | 82.00±15.42[a] | 89.66±14.15[a] | 122.00±66.19[a] | 99.00±29.02[a] |

Note: BMI = body mass index; SMM = skeletal muscle mass; BF% = body fat % by skinfold addition; WP = waist perimeter; AMAc = corrected arm-muscle area; TC = total cholesterol; TG = triglycerides; LDLc = LDL cholesterol; Glu = glucose; PVHIV = people living with HIV; THIV = time of HIV infection. *T-Test ** Mann-Whitney – p<0.05.

develop lipodystrophy, with a prevalence of lipoatrophy (33.1%), attributed to the use of PI and the time of use of ARVT[2,20].

Beraldo et al.[21] found a prevalence of lipodystrophy in 47.7% of patients with HIV, among whom 53% presented high abdominal adiposity, and more than 50% of those evaluated presented metabolic changes. In another study that included patients in a regimen with two types of protease inhibitors (PI), the mixed type of lipodystrophy was the most prevalent in 42.5% of patients, followed by lipoatrophy in 32.5%, and lipohypertrophy in 25%[22]. Our findings corroborate those previously been reported in the literature.

The use of anthropometric indicators is advantageous, simple, of good reliability and applicable on a large scale. Authors have shown high values of positive and significant correlation between anthropometric and image methods to measure body fat distribution in patients with HIV and concluded that the anthropometric model has advanced in the field of public health, facilitating early diagnosis and better management of lipodystrophy in patients with HIV, with precision and a diagnosis prediction of 80% in this target group[23].

In this study, based on the BMI, only men with HIV were diagnosed eutrophic, and the variables of central adiposity (WHR and WP) had significantly higher values among men without HIV, while for women, regardless of HIV, these measures seemed inadequate. A Brazilian study presented the indicators of central fat (WP and WHR) as the most efficient to identify metabolic changes in HIV-infected patients[21]. Sacilotto et al.[22] classified lipodystrophy into three subtypes according to the region of the body and metabolic changes and found that the group with lipohypertrophy presented greater changes, with higher values of total body fat per center, area of visceral fat, and BMI, compared to other groups with HIV.

In relation to BF%, individuals without HIV, regardless of sex, were classified with higher values in our study. In addition to increased body adiposity, there is speculation that people living with HIV have a greater probability of developing accelerated aging syndrome with musculoskeletal changes (lean mass and muscle function), aging approximately 15 years early. Thus, the use of methods that can measure body fat in a segmented way in these individuals is essential[21,22].

We observed, in relation to changes in body fat distribution by means of anthropometry, that men

**TABLE 3.** ASSOCIATION BETWEEN ANTHROPOMETRIC, BIOCHEMICAL PROFILE, TTO WITH AND WITHOUT SELF-REPORTED PRESENCE OF LIPODYSTROPHY OF PVHIV, ASSISTED BY SAE/STI/AIDS IN MACAÉ - RJ, 2018

| TTO TIME | | | | |
|---|---|---|---|---|
| With self-reported lipodystrophy | | | | |
| | 0\| - \|3 years | 3 - \|6 years | 6 - \|9 years | >9 years |
| BMI | 24.84± 3.56[a] | 31.88± 12.25[a] | 24.30±3.28[a] | 25.34±4.84[a] |
| WP | 88.55±9.15[a] | 103.56± 17.68[a] | 88.33±17.09[a] | 94.02±12.42[a] |
| SMM | 24.13±4.55[a] | 24.69±3.11[a] | 23.63±6.48[a] | 21.92±6.58[a] |
| AMAc | 34.31±16.96[a] | 48.47±14.73[a] | 44.81±24.72[a] | 41.19±11.55[a] |
| BF% | 24.92±5.63[a] | 24.65±5.03[a] | 20.81±1.58[a] | 24.96±3.57[a] |
| CT | 165.87± 58.49[a] | 244.20±68.96[a,b*] | 301.66±130.19[b*] | 228.37±54.03[a,b] |
| LDLc | 97.87± 40,55[a] | 163.20±64.33[b] | 163.50±6.36[b] | 142.00±36.64[a,b] |
| TG | 125.75± 63,12[a] | 147.60±48.78[a,b] | 262.00±51.18[b] | 167.50±69.03[a,b] |
| GLU | 103.00±44.86[a] | 104.20±7.85[a] | 92.33±4.16[a] | 101.11±28.72[a] |
| Without self-reported lipodystrophy | | | | |
| | 0\| - \|3 years | 3 - \|6 years | 6 - \|9 years | >9 years |
| BMI | 22.75±4.16[a] | 25.77±6.35[a] | 24.75±1.50[a] | 27.21±1.67[a] |
| WP | 83.60±11.88[a] | 96.00±13.85[a] | 91.12±7.23[a] | 100.16±5.37[a] |
| SMM | 26.95±7.15[a] | 23.25±8.50[a] | 21.07±2.88[a] | 24.64±3.01[a] |
| AMAc | 25.76± 4,06[a] | 38.61±20.72[a,b] | 36.02±5.56[a,b] | 47.40±6.03[b] |
| BF% | 25.48±5.24[a] | 27.09±3.62[a] | 24.86±5.30[a] | 23.45±4.98[a] |
| CT | 178.20± 31.77[a] | 228.66±45.08[a,b] | 214.50±62.20[a,b] | 243.80±17.92[b] |
| LDLc | 106.60± 24.54[a] | 149.00± 38.43[a] | 132.00±39.48[a,b] | 178.75±23.67[b] |
| TG | 96.40± 26.76[a] | 109.66±49.57[a,b] | 209.25±214.55[a,b] | 333.40±300.90[b] |
| GLU | 82.00±13.33[a] | 93.66±12.50[a] | 120.50±67.10[a] | 101.20±31.88[a] |

Note: BMI = body mass index; SMM = skeletal muscle mass; BF% = body fat % by skinfold addition; WP = waist perimeter; AMAc = corrected arm-muscle area; TC = total cholesterol; TG = triglycerides; LDLc = LDL cholesterol; Glu = glucose; PVHIV = people living with HIV; TTO = time of antiretroviral treatment. *T-Test ** Mann-Whitney – p<0,05.

with HIV presented a significantly greater loss of body fat and musculoskeletal mass, which is indicative of mixed lipodystrophy, and 77.7% of them confirmed, based on self-perception, lipoatrophy. In females, significant loss of body fat in the upper limbs, assessed by (DCT), stood out among those infected by the virus, which is indicative of lipoatrophy. However, regarding self-reports, most of them noticed mainly lipohypertrophy, although, in both sexes, mixed lipodystrophy was frequent in self-perception. The literature points to a recent study that compares self-reported signs (lipohypertrophy/lipoatrophy) with skinfold thicknesses in 815 people living with HIV. It found mainly an increase of fat in the neck and waist and a reduction of fat in the limbs[24], data similar to ours, and which demonstrate the importance of evaluating body redistribution in individuals with HIV per body segment.

In people infected by HVI, recognizing changes in body fat distribution and in the metabolic profile should be an important aspect to be addressed because the adipose tissue is an important trigger of metabolic alterations, raising the risk of morbidity and mortality, especially in patients under ARVT[2,3,20]. Beraldo et al.[21] found a high prevalence of lipodystrophy and metabolic changes in HIV patients under antiretroviral therapy, and Marins et al.[19] noticed that all people with HIV undergoing ARVT in his study presented changes in at least one type of lipid molecule in the lipid profile test, with emphasis on low levels of HDL and increased total cholesterol.

It is worth mentioning that, despite the adverse effects, ARVT is of paramount importance for the restoration of the immune system[22], and despite the relevance of the subject, no studies were found that correlated TTO and THIV with the presence or absence of lipodystrophy. However, our findings indicate that the time of ARVT negatively influenced the cholesterol profile, and there was mild impairment of muscle mass, according to the AMAc, in patients without self-reported lipodystrophy. The changes in body fat distribution, measured by anthropometry, were especially associated with the duration of HIV infection in individuals with self-reported lipodystrophy. Due to the use of ARVT in the long term, the need for studies exploring the association of antiretroviral therapy with lipodystrophy and biochemical profile in people living with HIV is imperative.

## CONCLUSION

The results of this study characterized changes in the behavior of the body fat distribution, known as lipodystrophy, which is associated with self-reported lipodystrophy, duration of treatment with antiretroviral drugs, and the duration of HIV infection.

### Contribution of the authors

LR SOARES, GC Menezes, APM Barreto participated in the research design, data collection, data analysis, discussion, and writing of the manuscript. NMA Cardoso, MSL Sant'Anna, JSR Casseb, and FLA Fonseca participated in the intellectual design of the manuscript, data analysis, data discussion, writing, and final revision of the manuscript. All authors declare there are no conflicts of interest.


## RESUMO

OBJETIVOS: *Indivíduos vivendo com HIV parecem mais propensos às alterações na redistribuição da gordura corporal, caracterizada como lipodistrofia, podendo acontecer em conjunto com as metabólicas. No presente estudo avaliaram-se tais impactos em adultos com e sem HIV e se associou ao tempo de diagnóstico do vírus e tratamento com antirretroviral.*

MÉTODOS: *Estudo tipo transversal, com 123 adultos, no qual 87 tinham HIV e 36 sem HIV, de ambos os sexos, em seguimento ambulatorial no Serviço de Atendimento Especializado (SAE) em Macaé - RJ. Foram feitos: 1) Alteração na distribuição da gordura corporal, mensurados por parâmetros antropométricos e lipodistrofia autorreferida; 2) Perfil bioquímico; 3) Associação entre tempo diagnóstico do HIV e tratamento com antirretroviral.*

RESULTADOS: *Incluíram-se 54,47% (n=67) do sexo masculino, 45,52% (n=56) do feminino, com média de idade de 37 anos. Destes, 87 eram pessoas vivendo com HIV, 29% (n=25) possuíam lipodistrofia autorreferida; tempo médio de infecção pelo vírus e tratamento antirretroviral (5,80±4,56 e 5,14±3,82 anos), respectivamente. Os pacientes com lipodistrofia autorreferida tiveram maior alteração na distribuição da gordura corporal entre 3-6 anos de diagnóstico do HIV e um perfil colesterolêmico negativo. O tempo de tratamento com antirretroviral influenciou o colesterol total e os triglicerídeos, mesmo para os pacientes sem lipodistrofia autorreferida, com mais de nove anos sob tratamento.*

CONCLUSÃO: *Neste estudo, o perfil colesterolêmico negativo se relacionou principalmente ao tempo de tratamento com antirretroviral, mesmo para os pacientes sem lipodistrofia autorreferida e as alterações na distribuição da gordura corporal, mensuradas por antropometria, se associaram especialmente ao tempo de infecção pelo HIV naqueles com lipodistrofia autorreferida.*


PALAVRAS-CHAVE: *Lipodistrofia. Perfil bioquímico. HIV.*

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ORIGINAL ARTICLE

# Application value of magnetic resonance hydrography of the inner ear in cochlear implantation

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## SUMMARY

OBJECTIVE: *This study aims to investigate the application value of magnetic resonance (MR) hydrography of the inner ear in cochlear implantation.*

METHODS: *146 patients were enrolled. MR hydrography and spiral CT examinations for the intracranial auditory canal were performed before surgery, and all imaging results were statistically analyzed in order to explore the application value of MR hydrography of the inner ear in cochlear implantation.*

RESULTS: *146 patients (292 ears) were examined. Among these patients, 13 were diagnosed with abnormal vestibular aqueducts (20 ears) by MR hydrography, while five were diagnosed with this disease by CT; 15 patients were diagnosed with inner ear malformation (19 ears) by MR hydrography, while 11 were diagnosed by CT (four were misdiagnosed); five patients were diagnosed with internal acoustic canal stenosis (eight ears) by MR hydrography, while two were diagnosed by CT (three were misdiagnosed); and four patients were diagnosed with cochlear fibrosis (five ears) by MR hydrography, while four were diagnosed by CT (four ears). The correct rate of diagnosis was 77.40% (113/146) based on CT, while the rate was 93.84% (137/146) based on MR hydrography.*

CONCLUSIONS: *MR hydrography imaging technique can be applied to the preoperative evaluation of cochlear implantation, providing accurate and reliable anatomic information on the inner membranous labyrinth and nerves in the internal acoustic canal and an accurate basis for the diagnosis of cochlear fibrosis and nerve development. This has a guiding significance for the selection of treatment schemes.*

KEYWORDS: *Magnetic Resonance Hydrography of Inner Ear Imaging; Cochlear Implants; Clinical Application Value.*

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## INTRODUCTION

Cochlear implantation (CI) is the most effective means of rehabilitation for patients with severe and very severe sensorineural hearing loss. Preoperative imaging examination is the basis for understanding the condition and characteristics of important anatomical regions related to surgery.[1] CI requires the surgeon to have enough and detailed understanding on the structures and pathological changes in the inner ear of patients, develop a comprehensive treatment scheme, and select the most suitable electronic cochlear implant and electrode before surgery.[4] Surgeons need to ensure the presence of inflammation and mastoid gasification, morphology, patency, and basal turn of the cochlea in the inner ear by imaging tests. However, due to limited density resolution, spatial resolution and other technical factors, traditional plain X-ray films and spiral CT (computerized tomography) examinations could not clearly and accurately display changes of the structure and function of the membranous labyrinth in the inner ear[5], and could not provide accurate and reliable information for the preoperative evaluation of CI.

MR hydrography images use the static or slowly flowing fluids in the body, so the imaging mainly consists of fluid-containing structures. MR hydrography exhibits hyperintense signals mainly through the T2 relaxation values of water, while the T2 relaxation values of the surrounding tissues show hypointense signals. Thus, this improves the resolution of the MRI device on tissues. The T2 relaxation value of water is higher than that of other tissues in the human body, and the T2WI sequence where the weight of T2 is very heavy can make the transverse magnetization vector of other tissues close to the total loss. Hence, signals could not be collected, while the long T2 relaxation value of water can maintain a large value of the transverse magnetization vector. Therefore, the signals collected by MRI are mainly from water structures with large transverse magnetization vector values.[6] This is the hydrography (water imaging) technology. Compared with MRI, it does not need direct or intravenous contrast, and it is very suitable for the imaging of fluid-containing structures [6].

Magnetic resonance (MR) hydrography of the inner ear adopts special imaging technology that focuses on signals on the water to clearly display the fine and complex structures contained in it, such as the internal labyrinth structure, internal acoustic canal, and vestibulocochlear nerve. Hence, it is of great value for the preoperative evaluation of CI.[2] Conventional cochlear implant is a surgical procedure performed on the posterior tympanum through a transmastoid facial recess approach that opens the facial nerve recess and exposes the round window niche and round window membrane. Finally, the electrode is implanted into the cochlea tympanic canal. On account of narrowness and large variations in the anatomical structure of the facial recess, it frequently presents tremendous difficulties and increasing lesion risks to the tympanum and facial nerves. Therefore, the observation and assessment by preoperative fossa temporal scanning are of great significance. Prof. Xuebin He et al. measured facial recess sizes and made a comparative analysis based on anatomical data [15]. The results revealed there was no statistical variation between them. It is deemed that the fossa temporal contributed to preoperative observing and measuring the facial recess size; the structures involved were the pyramid segment of the facial nerve, posterior wall of external auditory foramina, fenestra cochleae, and osseous labyrinth, which in the normal inner ear could be vital anatomic landmarks for operation reference.

However, despite the complicated and meticulous structures of the osseous labyrinth in the normal inner ear and other nerves as well as its deep position in the petrous bone, it is difficult to identify and probe the lesions in detail. The effects of conventional CT detection are not acceptable for diseases in the internal ear, including cochlear fibrosis, congenital malformation, and neuropathy. We have been trying to use 3D-FIESTA-C high spatial resolution and SNC (Sun Nuclear Corporation) as well as clear images of blood vessels and nerves, to measure the facial recess size associated with HDCT (high definition computerized tomography) scan. It not only clearly displayed both the inner ear labyrinth artery and nervus vestibulocochlear but also revealed the association between them.[3]

In this study, the application value of MR hydrography of the inner ear in cochlear implantation was evaluated. Details are reported as follows.

## METHODS

### Clinical data

After screening, 146 patients who underwent CI in our hospital from January 2012 to January 2014 were enrolled in this study. Among them, 85 were male, and 61 were female; their ages ranged from eight months

to 49 years (average: 6.5 ± 1.6 years). Among these patients, 112 had congenital sensorineural hearing loss, nine had drug-induced deafness, 15 had postlingual deafness, and 10 had unexplained deafness. All patients were confirmed with bilateral severe sensorineural hearing loss by audiological examination and received CI. Furthermore, 82 patients chose implantation on the right side, and 64 chose it on the left side. Moreover, 68 patients were implanted with a Nucleus 24M contour electrode, 21 with a 90K artificial cochlea (AB, USA), 25 with a Combi-40 cochlear implant (Med-EL), 16 with a Pusal implant, and 32 with a CS-10A implant (Nuoerkang).

## Experimental methods

Data were collected before surgery. Routine audiological examinations were initially performed: all patients underwent brainstem auditory-evoked potentials (BAEP), otoacoustic emission (OAE), acoustic impedance, and auditory steady-state response (ASSR); and the 40-Hz auditory event related potential (AERP) and brainstem electric response audiometry (BERA) could be additionally performed. Adult patients with postlingual deafness needed to undergo BERA independently. Audiological examinations revealed that all patients had preoperative ≥95 d Bn HL, and did not elicit bilateral OAE, while some patients elicited ASSR.

Next, MR hydrography was performed. Patients were placed in the supine position, the skull was placed in the middle, and both ears were kept symmetrically. Children with good cooperation did not require special surgeries. For infant patients, doctors asked the family to help the infants to fall asleep more easily or gave the infant sedation before the examination. Examinations were performed using the 3.0T GE Signa HDx MRI scanner in our hospital. The head of the patient was fixed with an 8-channel head array coil for MRI. The range of scanning included the petrous part and the head of the temporal bone. A three-dimensional fast advanced spin-echo (FASE) sequence was used to scan the region of interest transversely. The parameters of hydrography scanning of the inner ear were set as follows: slice thickness was set at 0.8 mm, the gap between slices at 0.4 mm, the number of slices at 64, TE at 2.5 ms, TR at 6.5 ms, FOV at 16×16 mm, and the scan matrix was set at 512×256. MRI image data were acquired twice. The original MRI images of the temporal bone were three-dimensionally reconstructed using an image processing software with maximum intensity projection (MIP). Next, the MIP hypointense signals of the surrounding tissues around the inner ear were cleared off using the workstation software. Finally, only the image of the region of interest was retained. The three-dimensional hydrography images of the inner ear in multiple axial planes were acquired by rotating the images 30-180°.

Thin-slice CT scanning of the temporal bone was performed using the GE Hi-speed spiral CT in our hospital. More attention was given to the patient's vestibular aqueduct, facial nerve, semicircular canal, vestibule, and cochlea during scanning. Scanning parameters were set as follows: slice thickness was set at 1.0 mm, the gap between slices at 1.0 mm, tube voltage at 120 kV, tube current at 200 mA, window width at 3,500 HU, and the window was set at 500 HU.

## Diagnostic criteria

### MRI diagnostic criteria

(1) Large Vestibular Aqueduct Syndrome

Reflected by the enlargement of the saccus lymphaticus, i.e., the maximum width of the midpoint of the internal part of the endolymphatic sac bone greater than 1.5 mm.

(2) Cochlear malformation

The absence of the cochlea, vestibule, or semicircular canal is reflected by the hyperintensity in the imaging.

(3) Narrowed internal auditory canal

Reflected by the narrow diameter of the internal auditory canal, which is < 2 mm.

(4) Fibrosis of the cochlea

Reflected by the decreased hyperintensity and the asymmetrical signal of the cochlea.

### CT criteria

(1) Large Vestibular Aqueduct Syndrome

Reflected by the enlargement of the middle of the vestibular aqueduct (greater 2 mm).

(2) Cochlear malformation

The absence of the cochlea, vestibule, or semicircular canal is reflected by the high density in the imaging.

(3) Narrowed internal auditory canal

Reflected by the narrow diameter of the internal auditory canal, which is < 2 mm.

(4) Fibrosis of the cochlea

Reflected by the increased density of the cochlea.

### Statistical processing

Statistical analysis was performed using IBM SPSS19.0 statistical software. Measurement data were expressed as mean ± standard deviation ($\bar{x}$ ± SD) and were evaluated using the *t*-test. Count data were evaluated using *the* $X^2$-test. The inspection level was set as α=0.05. *P*<0.05 was considered statistically significant.

### Results

Among the 146 patients (292 ears), 95 were confirmed with abnormal ears by surgical treatment. Among these, 13 patients were diagnosed with abnormal vestibular aqueducts (20 ears) by MRI, while five were diagnosed with this disease by CT; 15 patients were diagnosed with inner ear malformation (19 ears) by MR hydrography, while 11 were diagnosed by CT (four were misdiagnosed); five patients were diagnosed with internal acoustic canal stenosis (eight ears) by MR hydrography, while two were diagnosed by CT (three were misdiagnosed); and four patients were diagnosed with cochlear fibrosis (five ears) by MR hydrography, while four were diagnosed by CT (four ears). The correct rate of diagnosis was 77.40% (113/146) based on CT, while this rate was 93.84% (137/146) based on MR hydrography.

### Comparison of the results of spiral CT and MR hydrography in all patients

Detection results of abnormal ears by spiral CT, MR hydrography, and combined examination were acquired and compared. The results are shown in Table 1.

Table 1 shows that the differences in detection rates between spiral CT and MR hydrography, and between spiral CT and combined examination, were statistically significant (*P*<0.05). In addition, the MRI differences in the detection rate between MRI and CT, or between MRI and combined examination, were not statistically significant (*P*>0.05).

### Comparison of the detection results of abnormal ears by CT and MRI

Imaging detection results of abnormal ears by CT and MRI were collected and compared. These results are shown in Figure 1. Figure 1 shows that the differences in detection rates between MRI and spiral CT, and between combined examination and spiral CT, were statistically significant (*P*<0.05).

### Comparison of detection rate of cochlear malformation and inner ear canal stenosis by CT and MRI

According to the results of both imaging methods, the detection rates of cochlear malformation and inner ear canal stenosis were collected. The results are shown in Table 2. Table 2 shows that the difference in the diagnostic accuracy of cochlear malformation and stenosis of the inner ear canal between CT and MRI was statistically significant (*P*<0.05).

## DISCUSSION

The internal tissues of the membranous labyrinth in the human inner ear are full of lymph, and the internal acoustic canal is full of cerebrospinal fluid. Therefore, the detection of internal tissues of the membranous labyrinth and internal acoustic canal in the inner ear is strongly sensitive using MR hydrography. Hence, this can collect strong signals and is not affected by hypointense signals from the surrounding bony structure[7], thereby allowing the clear and accurate acquisition of images.

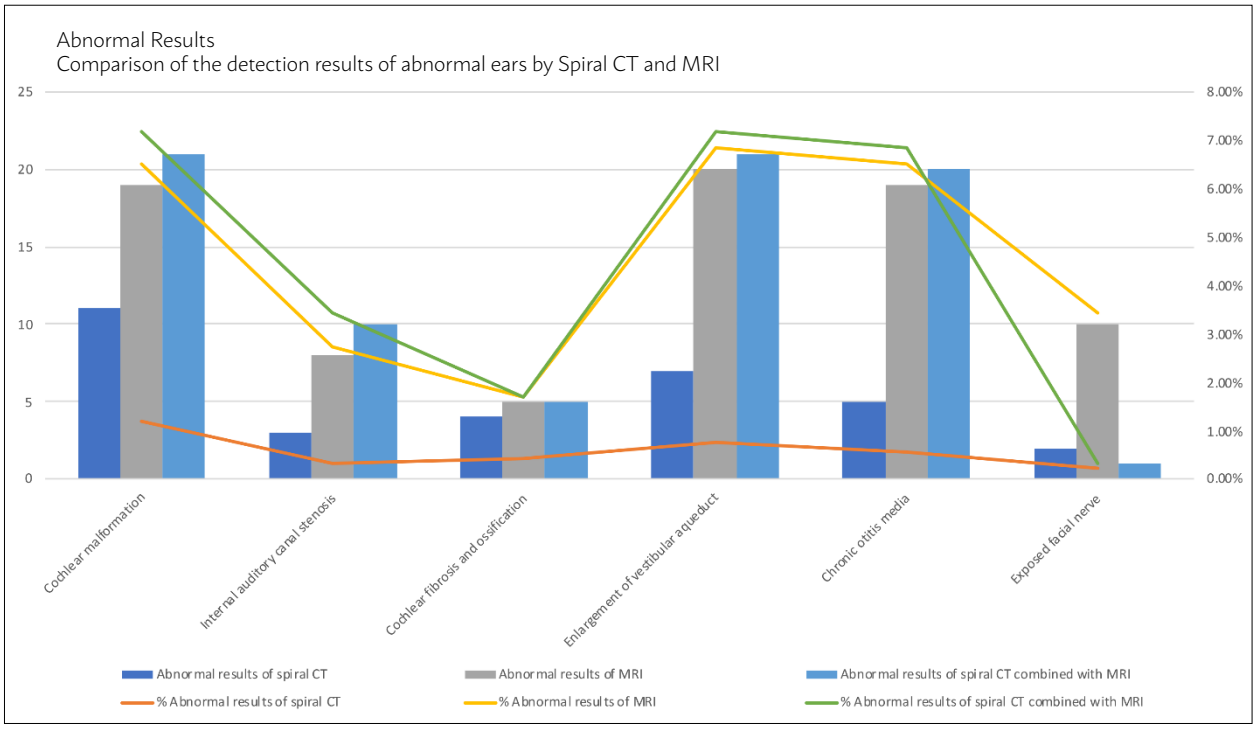
**TABLE 1.** COMPARISON OF THE RESULTS OF SPIRAL CT AND MR HYDROGRAPHY IN ALL PATIENTS

| Imaging manifestations | Abnormal results of spiral CT | | Abnormal results of MR hydrography | | Abnormal results of spiral CT combined with MR hydrography | |
|---|---|---|---|---|---|---|
| | Cases (ear) | Rate (%) | Cases (ear) | Rate (%) | Cases (ear) | Rate (%) |
| Cochlear malformation | 11 | 3.77 | 19 | 6.51 | 21 | 7.19 |
| Internal auditory canal stenosis | 3 | 1.03 | 8 | 2.74 | 10 | 3.42 |
| Cochlear fibrosis and ossification | 4 | 1.37 | 5 | 1.71 | 5 | 1.71 |
| Enlargement of the vestibular aqueduct | 7 | 2.40 | 20 | 6.85 | 21 | 7.19 |
| Chronic otitis media | 5 | 1.71 | 19 | 6.51 | 20 | 6.85 |
| Exposed facial nerve | 2 | 0.68 | 10 | 3.42 | 1 0 | 3.42 |
| Total abnormal ears | 31 | 10.62 | 81 | 27.74 | 87 | 29.79 |

Note: Rate = abnormal ears / total ears; Degree of freedom = 3

It was found that the preoperative imaging test is the only method that can objectively evaluate the conditions and characteristics of the essential anatomic regions related to surgery because accurate imaging of the structures and functions of the membranous labyrinth in the inner ear helps the surgeon select the most appropriate treatment and artificial cochlea electrode[8]. Among these, the test results of acute and chronic OM, internal acoustic canal malformation, cochlear malformation, and fibrous ossification can provide the most accurate indications for the surgeon. Spiral CT has a higher resolution in bone structure and ossification; therefore, its detection rates of bony development and lesions in the inner ear are high[9]. However, limited by density resolution, spiral CT could only acquire images of bony lesions in the bony labyrinth and internal acoustic canal; it has difficulty in acquiring images of soft tissue, cochlear membranous labyrinth, and fibrosis. Since the membranous labyrinth's internal acoustic canal in the inner ear are full of lymph fluid and cerebrospinal fluid, the MR hydrography technique could accurately evaluate structural changes in the membranous labyrinth and internal acoustic canal by acquiring the images of the shapes and distribution of the lymph fluid inside and outside[10]. Specific MRI signs could be used for the preoperative evaluation of surgical indications: under normal condition, MRI image of the internal acoustic canal exhibits cone- and tube-shaped hyperintense signals and hyperintense cerebrospinal fluid signals. The courses and gaps of vessels and nerves are definite. The cochlea exhibits a typical spiral snail-like shape, and the alveus utriculosus and saccule are integral, exhibiting a hyperintense signal. The semicircular

**FIGURE 1.** $X^2$-TEST OF THE DETECTION RESULTS OF ABNORMAL EARS BY CT AND MRI.




The inspection level was set at $\alpha$=0.05. $P$<0.05 was considered statistically significant; Degree of freedom = 3

**TABLE 2.** COMPARISON OF THE DETECTION RATE OF COCHLEAR MALFORMATION AND INNER EAR CANAL STENOSIS BY CT AND MRI

| Imaging manifestations | Abnormal results of spiral CT (percentage in Abnormal results of spiral CT combined with MR hydrography) | Abnormal results of MRI (percentage in Abnormal results of spiral CT combined with MR hydrography) | Total number of abnormal ears detected by spiral CT combined with MR hydrography | $\chi 2$ | P |
|---|---|---|---|---|---|
| Cochlear malformation | 11 (52.38%) | 19 (90.48%) | 21 | 145.326 | < 0.001 |
| Internal auditory canal stenosis | 3 (27.27%) | 8 (72.73%) | 11 | 5.699 | 0.019 |

duct exhibits a C shape, and its margins are smooth.[11] Under pathological conditions, the MRI performance of dysplasia and malformation of the inner ear is the absence of corresponding images. Generally, these were Michel and Mondini malformations.[12] The vestibular aqueduct syndrome mostly exhibits as abnormal hyperplasia of the endolymphatic duct and saccus lymphaticus, which shows hyperintense signals. Furthermore, attention should be paid in examining the cochlear nerve during the preoperative examination. A clinical trial confirmed that lesions in the cochlear nerve were the absolute contraindication in CI.[13] Spiral CT could not accurately evaluate whether the structure of the cochlear nerve changes, while the correct diagnostic rate of MR hydrography for cochlear nerve lesions is relatively high, showing the changes of the neural structure in the internal acoustic canal.[14] This has important significance for determining surgical indications.

MRI demonstrates the shape of the inner ear screened by the water composition. It is confirmed that the observed effect by MRI is worse than CT; while referring to the detection of mastoiditis, MRI is similar to CT. The comprehensive imaging of both endolymph and perilymph was captured by three-dimensional reconstructed MRI, without the bone composition. It is equivalent to three-dimensional reconstruction imaging by CT, excluding subjective dependence. The display of the cochlear duct, scala vestibule, and semicircular ducts is much better than CT. Besides, MRI is much more sensitive to detect the effect of fibro ossification of the internal and accurate to estimate pathological lesions than CT. Therefore, we believe that the three-dimensional reconstruction imaging evaluation method by MRI for use concerning the inner ear provided many more advantages compared to the single three-dimensional reconstruction by CT. In addition, the preoperative assessment provided a necessary reference value as a surgical supplement.

The limitation of the study is mainly its relatively small sample size since there are many kinds of abnormalities. A larger sample size can lead to more important results and conclusions.

## CONCLUSION

In summary, the MR hydrography technique can be applied to the imaging evaluation before CI, providing accurate and reliable anatomic information regarding nerves in the membranous labyrinth and internal acoustic canal of the inner ear. Furthermore, it provides an accurate basis for the diagnosis of cochlear fibrosis and nerve development and has a guiding significance for the selection of treatment schemes.

### Funding

Research project of Heilongjiang provincial health and Family Planning Commission(2016-541)

### List of Abbreviations

Magnetic Resonance: MR
Cochlear Implantation: CI
Otoacoustic Emission: OAE
Auditory Steady-State Response: ASSR
Auditory Event-Related Potential: AERP
Brainstem Electric Response Audiometry: BERA
Fast Advanced Spin Echo: FASE
Sensorineural Hearing Loss: SNHL

### Author Contributions

All authors contributed equally to the work.

## RESUMO

OBJETIVO: *Este estudo visa investigar o valor da aplicação da hidrografia por ressonância magnética (RM) do ouvido interno no implante coclear.*

MÉTODOS: *Cento e quarenta e seis pacientes foram inscritos. Os exames da hidrografia por RM e do CT espiral para o canal auditivo intracraniano foram executados antes da cirurgia, e todos os resultados da imagem foram analisados estatisticamente, a fim de explorar o valor da aplicação da hidrografia por RM do ouvido interno no implante coclear.*

RESULTADOS: *Centro e quarenta e seis pacientes (292 ouvidos) foram examinados. Dentre esses pacientes, 13 foram diagnosticados com aquedutos vestibulares anormais (20 ouvidos) pela hidrografia por RM, enquanto cinco pacientes foram diagnosticados com esta doença pelo CT; 15 pacientes foram diagnosticados com malformação do ouvido interno (19 ouvidos) pela hidrografia por RM, enquanto 11 pacientes foram diagnosticados por CT (quatro foram diagnosticados erroneamente); cinco pacientes foram diagnosticados com estenose de canal acústico interno (oito ouvidos) pela hidrografia por RM, enquanto dois pacientes foram diagnosticados por CT (três foram diagnosticados erroneamente); e quatro pacientes foram diagnosticados com fibrose coclear (cinco ouvidos) pela hidrografia por RM, enquanto quatro foram diagnosticados por CT (quatro ouvidos). A taxa correta de diagnóstico foi de 77,40% (113/146) com base no CT, enquanto a taxa foi de 93,84% (137/146) com base na hidrografia por RM.*

CONCLUSÕES: *A técnica de imagem da hidrografia por RM pode ser aplicada à avaliação pré-operatória do implante coclear, que pode fornecer informações anatômicas precisas e confiáveis sobre o labirinto membranoso interno e os nervos no canal acústico interno, além de uma base exata para o diagnóstico da fibrose coclear e do desenvolvimento do nervo. Isso tem um significado orientador para a seleção de esquemas de tratamento.*

PALAVRAS-CHAVE: *Hidrografia por ressonância magnética da imagem do ouvido interno. Implantes cocleares. Valor da aplicação clínica.*

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REVIEW ARTICLE

# Non-alcoholic fatty liver disease in patients infected with human immunodeficiency virus: a systematic review

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## SUMMARY

OBJECTIVE: *To evaluate the prevalence of nonalcoholic fatty liver disease (NAFLD) in patients with HIV/AIDS.*

METHODS: *The systematic review included articles indexed in MEDLINE (by PubMed), Web of Science, IBECS, and LILACS. Studies eligible included the year of publication, diagnose criteria of NAFLD and HIV, and were published in English, Portuguese, or Spanish from 2006 to 2018. The exclusion criteria were studies with HIV-infection patients and other liver diseases. Two reviewers were involved in the study and applied the same methodology, according to PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses).*

RESULTS: *One hundred and sixteen papers were selected, including full articles, editorial letters, and reviews. Twenty-seven articles were excluded because they did meet the inclusion criteria. A total of 89 articles were read, and 13 were considered eligible for this review. Four case series used imaging methods to identify NAFLD, and nine included histology. The prevalence of NAFLD in HIV-patients ranged from 30%-100% and, in nonalcoholic steatohepatitis (NASH), from 20% to 89%. A positive association between dyslipidemia, insulin resistance, and body mass index was observed. There was no agreement between the studies that evaluated the relationship between antiretroviral drugs and NAFLD.*

CONCLUSION: *This systematic review showed a high prevalence of NAFLD in HIV-patients, which was associated with metabolic risk factors. The possible association between antiretroviral therapy and NAFLD needs further studies.*

KEYWORDS: *Non-alcoholic fatty liver disease. HIV infections. Acquired Immunodeficiency Syndrome.*

## INTRODUCTION

Acquired Immunodeficiency Syndrome (AIDS) is caused by a retrovirus of the lentivirus family. This virus is transmitted mainly by sexual contact, parenterally, or through vertical transmission in infected pregnant women. According to the Joint United Nations Program on Human immunodeficiency virus (HIV)/AIDS (2016), there were 36.7 million individuals living with HIV worldwide. Access to antiretroviral

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therapy (ART) led to an increase in life expectancy, and it was estimated that 5.8 million people over the age of 50 years were living with HIV in 2015[1].

Liver diseases are a frequent cause of death unrelated to AIDS in these individuals. Abnormal liver enzymes are common in HIV-infected patients, even in the absence of other causes of liver disease, such as viral hepatitis or alcohol abuse. Co-infection with the hepatitis C virus (HCV) is very common, justifying the expressive relationship between HIV and liver disease deaths. However, NAFLD or NASH also has been reported in patients with HIV. These patients have presented an elevated prevalence of NAFLD/NASH and clinical manifestations of this liver disease[2].

The prevalence of NAFLD around the world is estimated in 25-30% of the population[3]. NAFLD has a broad spectrum, including hepatic steatosis and NASH, with the potential for progression to fibrosis, cirrhosis, and hepatocellular carcinoma[2,3]. Obesity is the main risk factor, and, therefore, NAFLD has become frequent in all populations[4,5].

In patients with HIV, as in the general population, NAFLD is associated with increased waist circumference (WC), low HDL levels, high triglyceride levels, and insulin resistance. Moreover, the potential impact of metabolic factors combination with antiretroviral therapy or direct HIV effects on the emergence of NAFLD needs to be evaluated[6].

This systematic literature review evaluated the prevalence of NAFLD in patients with HIV/AIDS

## METHODOLOGY

The review included articles indexed in the MEDLINE (through access to the PubMed), SciELO, IBECS, and LILACS databases. A search was also done based on the references of the articles found. Articles published in English, Portuguese, or Spanish were included from 2006 to 2018.

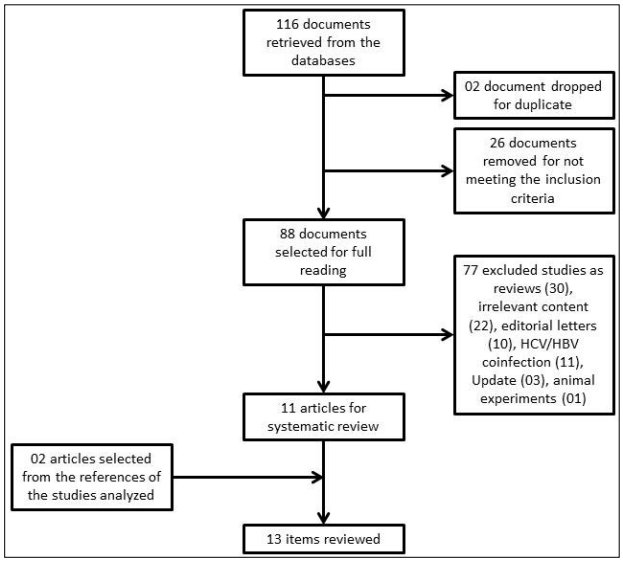
The descriptors were initially checked on the Virtual Health Library website (http://decs.bvs.br/) and the National Center for Biotechnology Information (https://www.ncbi.nlm.nih.gov/mesh) in order to use the most appropriate terminology to exclude other diseases that occur with hepatic steatosis. The descriptors "Nonalcoholic Fatty Liver Disease" and "HIV" were crossed using the Boolean operator "AND" through the Medical Subject Heading (MeSH) interface. Eligible studies included cross-sectional studies in humans. Review articles, updates, case reports, editorial letters, studies with HBV/HCV co-infected patients, and experimental studies were excluded. Two reviewers participated in the study using the same methodology according to *the Preferred Reporting Items for Systematic Reviews and Meta-Analyseses* (PRISMA)[7].

## RESULTS

One hundred and sixteen papers were selected, including full articles, editorial letters, and reviews. After applying the filters for time, languages, and delimitation of studies in humans, 27 documents were excluded because they did not fit into the inclusion criteria, or they were duplicated. A total of 89 articles were read, and 77 documents were excluded: 30 review articles, 22 with content irrelevant to the objective of this study, 10 editorial letters, and 11 articles involving HIV/HCV/HBV coinfection studies, three *updates*, and one experimental study. Two articles were added after a search based on the bibliographic references. Thirteen articles were included in this review (Figure 1).

Table 1 shows the prevalence of NAFLD and NASH in 11 and 12 studies, respectively. Just over half of the studies were conducted in the USA and Canada[2,8-13]. The three largest series used imaging tests, such as computed tomography (CT) or ultrasonography (USG) or Magnetic resonance to identify hepatic steatosis[11,14-16]. The smaller series used hepatic biopsy, which is considered a gold standard for the diagnosis and staging of NAFLD/NASH[2,8-10,12,13,17,18].

**FIGURE 1.** ALGORITHM OF ARTICLES SELECTION.



The prevalence of NAFLD in the HIV-positive population ranged from 30% to 100% and NASH from 20% to 89%.

The study by Price et al.[19] was not considered to assess prevalence due to the selection of 719 patients without distinction from patients coinfected with HBV or HCV. However, after adjusting for the NAFLD confounding variables, this study was included in Table 2, which specifically assessed the associated factors.

The selection of patients in the many studies did not occur in the same way, and this aspect directly interfered with the prevalence findings. Figure 2 shows the studies according to the sample selection method.

The evaluation of the main variables studied in 11 of 13 studies can be identified in Table 2. A positive association was found for insulin resistance (IR) or diabetes mellitus (DM) in six of seven studies[6,8,15-17,19],

**TABLE 1.** PREVALENCE OF NONALCOHOLIC FATTY LIVER DISEASE AND NONALCOHOLIC STEATOHEPATITIS IN PATIENTS INFECTED WITH HIV ALONE

| Reference | Country | Diagnostic Method | N | Prevalence | |
|---|---|---|---|---|---|
| | | | | NAFLD | NASH |
| Perez et al., 2018 [14] | Spain | Magnetic resonance | 72 | 33,3% | - |
| Morse et al., 2015 [8] | USA | Biopsy | 62 | 73% | 54% |
| Vodkin et al., 2015 [2] | USA | Biopsy | 86 | 38% | 24% |
| Nishijima et al., 2014 [15] | Japan | Ultrasonography | 485 | 31% | - |
| Rivero-Juarez et al., 2013 [17] | Spain | Biopsy | 10 | 100% | 20% |
| Sterling et al., 2013 [9] | USA | Biopsy | 14 | 64% | 28% |
| Arendt et al., 2011 [10] | Canada | Biopsy | 20 | 30% | 70% |
| Crum-Cianflone et al., 2009 [11] | USA | Ultrasonography | 216 | 31% | 20%* |
| Ingiliz et al., 2009 [18] | France | Biopsy | 30 | 60% | 89% |
| Akhtar et al., 2008 [12] | USA | Biopsy | 23 | 56% | 39% |
| Guaraldi et al., 2008 [16] | Italy | Computer Tomography | 225 | 36% | - |
| Mohammed et al., 2007 [13] | Canada | Biopsy | 26 | 100% | 55% |

* The study by Crum-Cianflone used biopsies from 55 patients. NAFLD: nonalcoholic fatty liver disease; NASH: nonalcoholic steatohepatitis; HIV: human immunodeficiency virus

**TABLE 2.** CLINICAL CONDITIONS AND LABORATORY ABNORMALITIES ASSOCIATED WITH NONALCOHOLIC FATTY LIVER DISEASE IN MONO-INFECTED HIV PATIENTS.

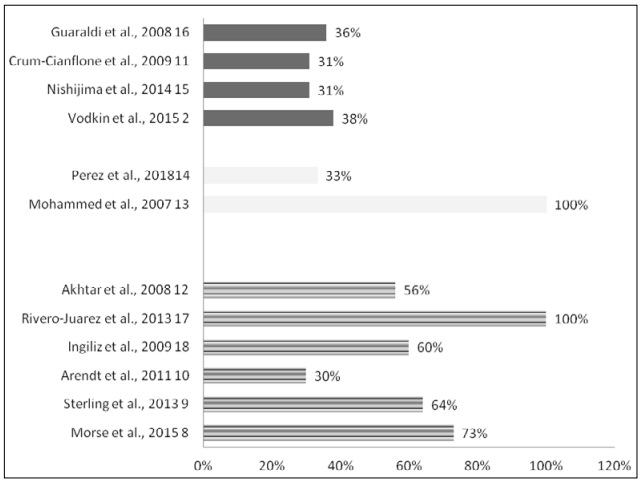
| Reference | Country | Diagnostic Method | N | Clinical Conditions and Laboratory changes |
|---|---|---|---|---|
| Morse et al., 2015 [8] | USA | Biopsy | 62 | IR/DM (+); Hepatic steatosis (+); Aminotransferases elevation (+); Polymorphyms PNPLA3. |
| Vodkin et al., 2015 [2] | USA | Biopsy | 86 | Dyslipidemia (+); aminotransferases elevation (+); Canalicular enzymes (+); ART (-); HIV (+) |
| Nishijima et al., 2014 [15] | Japan | Ultrasonography | 485 | IR/DM (-); BMI/WC (+); Dyslipidemia (+), hypertriglyceridemia and elevated LDL levels; aminotransferases elevation (+), elevated ALT; ART (-);Elevated CD4+ (+) |
| Price et al., 2014 [19] | USA | Computer Tomography | 719 | IR/DM (+); BMI/WC (+); Elevation of aminotransferases (+), elevated ALT; ART (+), greater cumulative exposure to Dideoxynucleoside analogues (Stavudina, Didanosine, Zalcitabine); HIV (-); CD4+ (-) |
| Rivero-Juarez et al., 2013 [17] | Spain | Biopsy | 10 | IR/DM (+); BMI/WC (+); Dyslipidemia (+), hypertriglyceridemia; Hepatic steatosis (+); ART (-); CD4+ plus hypertriglyceridemia (+) |
| Sterling et al., 2013 [9] | USA | Biopsy | 14 | IR/DM (+); Canalicular enzymes (+) plus hypertriglyceridemia |
| Crum-Cianflone et al., 2009 [11] | USA | Ultrasonography | 216 | BMI/WC plus Hypertriglyceridemia (+); Dyslipidemia (+), hypertriglyceridemia and decreased HDL levels; ART (-); HIV (-); CD4+ (-) |
| Ingiliz et al., 2009 [18] | France | Biopsy | 30 | IR/DM (+); BMI/WC (-); Dyslipidemia (-); ART (-); HIV (-) |
| Akhtar et al., 2008 [12] | USA | Biopsy | 23 | BMI/WC (+); ART (+), all patients who presented DHGNA were exposed to NRTIs; HIV (+) |
| Guaraldi et al., 2008 [16] | Italy | Computer Tomography | 225 | IR/DM (+); BMI/WC and Visceral Adipose Tissue (+); Dyslipidemia (+); aminotransferases elevation (+); ART (+), exposure to NRTIs |
| Mohammed et al., 2007 [13] | Canada | Biopsy | 26 | BMI/WC (-); Dyslipidemia (+), hypertriglyceridemia; |

(+): Associated factor present, (-): Associated factor absent; IR: insulin resistance; DM: diabetes mellitus; BMI: body mass index; WC: waist circumference; ART: antiretroviral therapy; NRTIs: Nucleoside Reverse Transcriptase Inhibitors; HIV: human immunodeficiency virus.

body mass index (BMI) or waist circumference (WC) in six of eight studies[11,12,15-17,19], dyslipidemia in six of seven studies; hypertriglyceridemia was present in all studies[2,11,13,15-17], and aminotransferases were elevated in five out of five studies[2,8,15,16,19].

Five of eight studies found a negative association between HART therapy and NAFLD/NASH[2,11,15-18]. A positive association was limited to only three studies, in which reverse transcriptase nucleotide inhibitors (NRTIs) were the most commonly reported cause of NAFLD[12,16,19]. The viral load also was not associated with NAFLD/NASH in three of five studies[10,18,19], and one study associated the prolonged duration of HIV infection with NAFLD/NASH[2]. All were cross-sectional studies.

**FIGURE 2.** PREVALENCE OF NONALCOHOLIC FATTY LIVER DISEASE ACCORDING TO THE SAMPLE SELECTION CRITERIA.




Patients with elevated aminotransferases; Patients with NAFLD; Randomly selected patients

## DISCUSSION

The present systematic review observed an elevated prevalence of NAFLD in HIV infected patients, which ranged from 30% to 100% of the cases. However, the selection of patients did not use similar methodologies. In several studies, the patients were diagnosed by imaging methods[10,15,16] and in others by liver biopsy. In randomized trials, less variation in the prevalence of NAFLD (31% to 38%) was observed.

Another factor that should be considered is the patients' selection for these studies. The samples from patients with HIV selected from the previous elevation of aminotransferases or after ruling out other causes of liver diseases could also influence the higher prevalence of NAFLD.

The liver biopsy is still the most effective method for staging NAFLD, diagnosing its various stages of steatosis, and differentiating NASH. However, it is considered an invasive method, is not free of complications, and has high costs.

The review has shown that MRI is more sensitive compared to USG to identify smaller amounts of fat[20]. The largest series in this review used imaging methods to diagnose steatosis[11,15,16]. Crum-Cianflone et al.[11] added liver biopsy to 55 patients who had abnormalities in USG and/or elevated liver enzymes. The results indicated a prevalence of NASH in these patients.

The prevalence of NAFLD was also documented in studies performed in the USA, Japan, and Italy, before the results reported for these 13 studies included in this review. In 2000, a study of 126 patients in the USA to evaluate possible living donors found 20% of steatosis greater than 30%[21]. A study conducted in Italy with 3,345 patients diagnosed 20% of NAFLD by USG in individuals without suspected liver disease[22]. In Japan, a cohort of 35,519 individuals showed an increase in NAFLD prevalence from 12.6% to 30.3% in 12 years[23]. These data then indicate a higher prevalence of NAFLD in HIV-infected individuals.

Some authors[6,18] suggested that insulin resistance (IR) was associated with antiretroviral therapy (ART), and others [9,16,19] hypothesize that IR may be a product of common lipodystrophy in HIV-infected patients in use of ART or that IR could also be associated with hypertriglyceridemia secondary to ART[17].

In some studies, on HIV-infected patients, NAFLD was associated with metabolic factors such as obesity and dyslipidemia, especially hypertriglyceridemia[13], and it also has been suggested that abdominal obesity is an important predictor of NAFLD. Central obesity has been related to elevated levels of adiponectin and leptin, and these cytokines have been involved with IR and hepatic steatosis.

Although most of the studies in this review have suggested that metabolic factors are relevant for the development of NAFLD/NASH in HIV-infected patient, the relationship of NAFLD in these patients with ART, mainly D analogs (didanosine/ddI, stavudine/d4T, and zalcitabine/ddC) have been discussed. All these drugs can promote hepatocyte mitochondrial toxicity, lactic acidosis, and hepatic steatosis. However, the results have been controversial[15,17,19].

This systematic review has some limitation: the causal relationships and natural history of HIV infection and NAFLD cannot be confirmed in these

patients because most studies were cross-sectional; the differences in sample selection and the method used for the diagnosis of NAFLD; and the difficulty in estimating the overall prevalence of NAFLD in patients with HIV.

## CONCLUSION

In conclusion, this review showed that there is a high prevalence of NAFLD in HIV patients; the HIV-infection treatment has increased the quality of life in these patients, although it also increased the prevalence of obesity and, consequently, NAFLD in this population. Metabolic factors are the most frequent risk factors of NAFLD in HIV patients, although a possible association between antiretroviral therapy and NAFLD has been suggested.

### Disclosure

The authors report no conflict of interest.

### Funding statement

The authors were responsible for the manuscript's financial support.

### Author Contributions

Conceived and designed the experiments: LBP, RR, CD, and HPC. Performed the experiments: LBP, RR. Analyzed the data: LBP, RR, CD, HPC. Wrote the paper: LBP, RR, DV, CD, VC, VC, VS, HPC.

## RESUMO

OBJETIVO: *Avaliar a relevância da doença hepática gordurosa não alcoólica (DHGNA) em pacientes com HIV / AIDS.*

MÉTODOS: *A revisão sistemática foi realizada utilizando instrumentos de busca de material científico indexado, incluindo MEDLINE (pela PubMed), Web of Science, IBECS e LILACS. Estudos elegíveis incluíram o ano de publicação, critérios para diagnostico de DHGNA e HIV, publicados em inglês, português e espanhol, entre 2006 a 2018. Os critérios de exclusão incluíram estudos com pacientes com outras doenças do fígado. Dois revisores foram envolvidos na pesquisa dos artigos e o PRISMA (Preferred Reporting Items for Systematic Reviews and Meta - Analyses) foi utilizado nas análises.*

RESULTADOS: *Cento e dezesseis artigos foram selecionados, 27 excluídos porque não preencheram critérios de inclusão e assim, 89 foram lidos pelos investigadores. Desses, 13 artigos foram incluídos na revisão. Quatro séries de casos utilizaram métodos por imagens para identificação de DHGNA e nove estudos utilizaram biópsia hepática. A prevalência de DHGNA em pacientes com HIV variou de 30% a 100% e esteato-hepatite não alcoólica (EHNA) entre 20% e 89%. Na avaliação das principais variáveis estudadas, observou-se a associação positiva entre dislipidemia, resistência à insulina e índice de massa corporal. Não houve concordância entre os artigos que avaliaram a relação dos antiretrovirais com a DHGNA.*

CONCLUSÕES: *A presente revisão sistemática sugere elevada prevalência de DHGNA em pacientes infectados com HIV. DHGNA nesses pacientes foi associada principalmente a fatores metabólicos. A possível associação entre terapia antiretroviral e DHGNA nesses pacientes vem sendo discutida, mas são necessários mais estudos para estabelecer essa associação.*

PALAVRAS-CHAVE: *Hepatopatia gordurosa não alcoólica. Infecções pelo HIV. Síndrome de Imunodeficiência Adquirida.*

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REVIEW ARTICLE

# Solid pseudopapillary neoplasia of the pancreas: a review

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**SUMMARY**

OBJECTIVES: *To review the literature and the diagnosis of conventional histopathological routine and immunohistochemistry of the cases diagnosed with Solid Pseudopapillary Neoplasm of the Pancreas (SPNP).*

METHODS: *The review of the literature was done using the Pubmed and solid Google-Scholar databases, through the historical, clinical aspects and diagnostic methods of SPNP. The review of SPNP cases diagnosed in the University Hospital Clementino Fraga Filho was carried out from 1977 to 2018.*

RESULTS: *Intratumoral phenotypic heterogeneity of SPNP was evidenced in the cases studied, taking into account macroscopic, microscopic, and immunohistochemical patterns.*

CONCLUSIONS: *The results show the importance of the examination of several fragments obtained from different regions of the neoplasia since not all of them present the same molecular alterations.*

KEYWORDS: *Pancreas. Solid Pseudopapillary Neoplasia.*

## INTRODUCTION

Solid pseudopapillary neoplasm of the pancreas is a rare tumor, with low potential of malignancy, of uncertain lineage, and favorable prognosis in most cases[1]. It has received different denominations, including "Frantz tumor", "cystic solid tumor", "papillary cystic tumor", "papillary epithelial neoplasia", among others. In 1996, it was defined by the WHO as a "solid pseudopapillary tumor" for the international histological classification of pancreas tumors[1]. That name covers the most distinct macroscopic and microscopic aspects of the neoplasia, i.e., solid and pseudopapillary. It represents around 1-3% of all exocrine pancreatic neoplasias [3]. It is most frequent in women (82%) of all ages. It is usually asymptomatic, but sometimes a palpable mass, pain, and abdominal discomfort, and

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nausea can be observed[5]. It is characterized by a solid-cystic growth pattern with pseudopapillary structures. Surgical resection is the treatment of choice and provides a good prognosis, even when there is distant metastasis or recurrence[1,6]. The is no apparent ethnic predilection or any association with known clinical or genetic syndromes, although some rare cases have been reported in patients with familial adenomatous polyposis (FAP)[7]. Due to its rarity, the clinical data regarding these tumors are, most often, limited to case reports or small series, especially in the Asian population[7]. However, the diagnosis for SPNP has been more frequent due to the awareness regarding its existence, to the more widespread use of immunohistochemical methods, and retrospective studies on tumors that were not properly identified[8].

Despite several studies using electron microscopy and immunohistochemistry, the cell origin of this neoplasia remains uncertain. Several researchers favor the hypothesis of a multipotential primitive cell as an origin, particularly due to the absence of a predominant line of differentiation and the multidirectional differentiation found[9]. In a recent study of 14 SPNP pediatric patients, no evidence was found of the PDX1, SOX9, PTF1A, and NKX2.2 transcription factors associated with pancreatic development[10]. An extrapancreatic origin has been suggested by some authors[6] due to several cases of a reported presence of primary tumors in different areas of the pancreas, such as the ectopic pancreas[11], retroperitoneum[12], gastroduodenal area [13], and ovary[14]. The origin of the primitive cells in the genital system, over a pancreatic origin, has been considered by some authors[15].

## CLINICAL ASPECTS

Solid pseudopapillary neoplasm of the pancreas is usually detected incidentally on routine physical examinations or abdominal imaging exams performed for various reasons[3]. The signs and symptoms are nonspecific and related to the intra-abdominal mass, including pain, dyspepsia, early satiety, nausea, and vomiting[1]. Jaundice is rare, even when the tumor is located at the head of the pancreas. The serological tumor markers are normal, and there is no description of association with functional endocrine syndromes[5]. Since surgical resection is usually curative, in most cases, and recurrences can be treated surgically, it is important to have an accurate diagnosis[6]. SPNP should be considered in the differential diagnosis of any solid or partially cystic mass, located in the pancreas or in the upper abdomen, mainly in young women[16].

In computed tomography, magnetic resonance, and ultrasound, the neoplasm is often well-circumscribed, encapsulated and heterogeneous, often with cystic and hemorrhagic areas and, at times, with calcifications[17]. The preoperative diagnosis can be established by means of fine-needle biopsy guided by endoscopic ultrasound (echoendoscopy). Echoendoscopy became very useful to assess pancreatic lesions observed in other imaging exams or when there is a suspicion of such injury based on clinical and laboratory examinations. The exam is generally safe and can be performed in most cases, and the material obtained must be evaluated by the cytopathologist through smears or cell blocks (*cell block*). The results allow for a treatment based on the diagnoses obtained. Its use allows surgeons and oncologists to have more appropriate planning for the patient's approach[18].

## ANATOMOPATHOLOGICAL DIAGNOSIS

SPNP can occur in any region of the pancreas, and, in general, one third occurs in the head, one third in the body, and another third on the tail. Macroscopic examination shows masses that vary from 0.5 cm to 25.0 cm in diameter (mean diameter of 8-10 cm). In general, they are rounded, well-circumscribed, and separated from the pancreatic parenchyma by a fibrous pseudocapsule; however, under microscopy, neoplastic cells can be seen infiltrating the pancreatic parenchyma, permeating acini, and pancreatic islets[5]. The cut surface shows variable appearance, with yellowish or brownish solid areas, hemorrhagic foci, or cystic degeneration filled with necrotic debris[5,8]. Smaller tumors tend to be more robust than those of larger diameter, and hemorrhagic-cystic areas, when extensive, may suggest a pseudocyst. They rarely spread to the stomach, the duodenum or to the spleen, and metastases occur in 5-15% of cases, mainly to the liver and peritoneum. The staging follows that of other pancreatic carcinomas[5].

The microscopic appearance of SPNP is heterogeneous, with a varied proportion of solid, pseudopapillary, hemorrhagic, and pseudocystic areas, representing the solid and cystic natures of the neoplasm[5,8]. The solid areas, located mainly in the periphery of the tumors, when these are notably

hemorrhagic-cystic, are formed by little cohesive cells, polygonal, monomorphic, with eosinophilic cytoplasm, or with a light or spumous appearance, separated by delicate blood vessels amidst a variable amount of perivascular collagen[5,8]. The pseudopapillary tumors are formed by the degeneration of the little-cohesive cells, leaving those who are closest to the conjunctive-vascular axis. These cells are frequently located perpendicularly to the axis, leaving the core in the apical position. The nuclei are rounded or oval, with disperse chromatin, and, at times, have longitudinal folds. Mitoses are rare (average of 0 to 10 in 50 fields of large magnification). Some of the neoplastic cells contain intracytoplasmic eosinophilic globules, positive to staining by PAS (Periódico-Schiff Acid), after digestion with diastole; these globules can also be found in the extracellular medium. Foci of calcification, foreign-body giant cells containing cholesterol crystals, and bizarre nuclei can also be observed[5]. Cellular pleomorphism and cell atypia are not common but have been reported, mainly in the more aggressive forms of neoplasia[19]. Perineural invasion, angioinvasion, and infiltration of the adjacent pancreatic parenchyma do not indicate a more aggressive behavior, since SPNPs without these characteristics can metastasize, which is why all these tumors are, therefore, classified as low-malignant neoplasms[5].

## IMMUNOHISTOCHEMISTRY

Histologically, the SPNP phenotype does not resemble any of the pancreatic epithelial cells[8], but its histological appearance is very characteristic and, in most cases, can provide a diagnosis; immunohistochemistry is used to confirm the diagnosis or, in some cases, to assist in the differential diagnosis[20]. A aberrant, nuclear, and cytoplasmic positive response to beta-catenin, the loss of membrane expression of E-cadherin[20], the characteristic perinuclear granular intracytoplasmic marking (dot-like) to CD99[21], associated with a positive response to the progesterone receptor[20], CD10 and CD56[22] constitute a basic immunohistochemical scenario for the histopathologic diagnosis of SPNP. In a study with 19 SPNPs, markers such as cytokeratins and alpha-1 antitrypsin were expressed in varying degrees, and chromogranin A had no expression[22]. The expression of Ki-67 in the usual forms of neoplasia is usually low; however, in aggressive forms, it can reach 50% of positivity [19].

## DIFFERENTIAL DIAGNOSIS

The histopathological diagnosis for this tumor is sometimes difficult, since its histomorphology and immunophenotype may suggest other exocrine and endocrine pancreatic tumors[7]. When in the SPNP there is a predominance of solid areas or light cells, or when there are pseudopapillary areas in neuroendocrine tumors, the immunohistochemical study is essential for the differential diagnosis, especially in specimens obtained by needle biopsy[20]. The solid pattern resembles that of acinar cell carcinoma and neuroendocrine tumor, while the cystic aspect is observed in pancreatic adenocarcinomas and neuroendocrine tumors. This should be the primary neoplasm to be excluded in the differential diagnosis, because in addition to the morphological similarity, the solid pseudopapillary pancreatic neoplasia can express some neuroendocrine markers in the immunohistochemistry, such as CD56, neuron-specific enolase, progesterone receptor and, more rarely, synaptophysin. However, the nuclear expression of beta-catenin, the loss of membrane E-cadherin, positive CD10, associated with the absence of chromogranin and perinuclear granular expression of CD99 favor the diagnosis of SPNP[3,7,20,21].

## MOLECULAR PATHOLOGY

Molecular analysis of SPNPs shows that they are distinct from pancreatic adenocarcinomas. Changes in genes *KRAS, CDKN2A/p16, TP53,* and *SMAD4/DPC4,* often present in the ductal carcinoma, have not been observed in SPNPs; however, almost all SPNPs feature somatic point mutations in exon 3 of *CTNNB1*, the gene that encodes beta-catenin[23]. These mutations are related to the activation of the Wnt/β-catenin signaling pathway, preventing the intracytoplasmic phosphorylation and the subsequent degradation of the beta-catenin protein, which then accumulates in the nucleus of neoplastic cells. As a result, 90% of SPNPs present an abnormal pattern of nuclear marking of the beta-catenin protein, while in the healthy pancreas, the marking is on the membrane. This nuclear accumulation of beta-catenin stimulates the transcription of several genes, such as *c-myc* and *cyclin D1*, both involved in cell proliferation[23]. In addition, β-catenin interacts with E-cadherin, so that the deregulation of the first also interferes in the expression of the second, and, as a consequence, no E-cadherin membrane expression is observed in most SPNPs[23]. The loss of the normal expression of E-cadherin seems to be related

to the lack of adhesion and cohesion of neoplastic cells among themselves, causing the typical pseudopapillary aspect of this neoplasm, like with the cystic degenerations observed in it[20]. In a study on methylation in three different areas of the same tumor, Chagas and col.[24] found methylation of codifying genes of the protein p16 (cyclin-dependent kinase inhibitor 2A) and TIMP-2 (tissue inhibitor of metalloproteinase 2) in two areas, indicating a potential for malignancy and heterogeneous progression in this neoplasia due to the inactivation of the expression of these genes. The protein p16 is an important tumor suppressor, reducing cell proliferation and nontissue inactivation of metalloproteinase 2, encouraging the degradation of the extracellular matrix and the invasion and the occurrence of metastases. In a molecular study of three distinct areas of the tumor were identified by mass spectrometry (MS) 1.427, 5.786, and 4.298 proteins, respectively, being 1.337 common to all three fragments, showing the heterogeneity of tumor[25].

## CASES REVIEWED IN 21 YEARS IN THE PATHOLOGICAL ANATOMY SERVICE OF THE UNIVERSITY HOSPITAL CLEMENTINO FRAGA FILHO - UFRJ

### Methodology

Were reviewed eight cases of SPNP diagnosed in the period of 21 years (1997-2018), in the HUCFF/UFRJ, of seven female patients aged between 12 and 46 years (project approved by the CEP HUCFF/UFRJ under CAE No. 64915717.0.0000.5257). We carried out a review of the medical records to retrieve the patients' clinical and evolution information, post-surgery. We observed that the main clinical manifestations reported were abdominal pain, more precisely in the right hypochondrium (three cases), and on the left (one case), nausea, and vomiting. The presence of a palpable abdominal mass was observed in four cases. Three patients whose neoplasias were located in the head of the pancreas were subjected to duodenopancreatectomy (Whipple surgery) and two to body-tail pancreatectomy and splenectomy (neoplasia located in the body-tail region of the pancreas). Three patients were diagnosed by echoendoscopic pancreatic biopsy, and one was later submitted to surgery.

In the review of medical records, we observed that a patient was followed-up on an outpatient basis for four years, another for two years, and a third is still being followed-up (P16 5242), without any complications in this period. In the medical records of four patients, no information was found regarding the period after discharge (Table 1).

The paraffin blocks corresponding to the examinations were obtained from the archive of the Pathology Service, HUCFF/UFRJ, and their respective histological sections were submitted to routine techniques for conventional histopathology and immunohistochemistry assays (Table 2). In one case, a molecular biology assay was conducted[24,25].

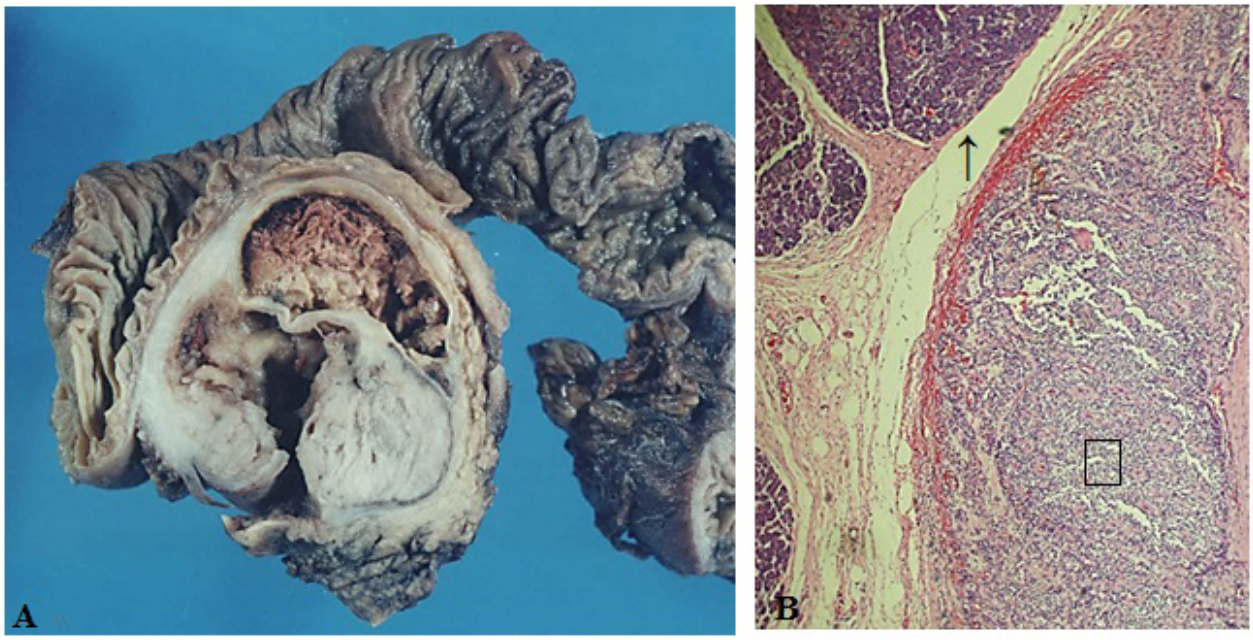


**FIGURE 1.**

**TABLE 1.** CASES REVIEWED IN TWENTY ONE YEARS IN THE ANATOMIC PATHOLOGY SERVICE - HUCFF

| Biopsy No | Age | Clinic | Location of the neoplasia | Dimen-sions in cm | Previous diagnosis | Procedure |
|---|---|---|---|---|---|---|
| B2116-97 | 27 | LH pain, LH palpable mass | Head and body | 10 x 7 x 6 | Cystadenoma, cystade-nocarcinoma a | Duodenopancreatectomy |
| P00 3249 | 46 | LH pain, palpable mass LH, vomiting | head | 6.5 x 6 x 4.5 | Adenocarcinoma | Duodenopancreatectomy |
| P11 1978 | 14 | Abdominal pain, vomiting | Body and tail | 2.5 x 2 x 2 | Pancreatoblastoma | Body/tail pancreatec-tomy |
| C12 1733 | 13 | Abdominal mass | Body and tail | NI | SPNP | Echoendoscopic biopsy |
| C15 1962 | NI | Abdominal pain | Body | NI | SPNP, NET | Echoendoscopic biopsy |
| C15 7785 (C15-1962) | 23 | LH pain, nausea, vomiting | Body and tail | 7 x 4 x7 | SPNP (previous cytologi-cal diagnosis) | Body/tail pancreatec-tomy |
| P16 5242 | 12 | LH pain, LH palpable mass, vomiting | Head | 6 cm of diameter | To be clarified | Duodenopancreatectomy |
| C18 113 | 34 | NI | Tail | 3 | Mucinous neoplasia | Echoendoscopic biopsy |

NI - no informatiion; LH; left hypochondrium; SPNP -solid pseudopapillary neoplasm of the pancreas; NET: neuroendocrine tumor ; HUCFF - University Hospital Clementino Fraga Filho

**TABLE 2.** HISTOPATHOLOGY

| Biopsy No | EC | PSP | LGT C | AP | DGC | HE | F | A | M | C |
|---|---|---|---|---|---|---|---|---|---|---|
| B2116-97 | + | + | + + | ++ | AEG | + | +++ | + | 0 | FI |
| P00 3249 | + | + | + + | + | CN | + + | ++ | - | 2 | F |
| P11 1978 | + | + | + | + | CN; C; GGCCC; GT | + + | ++ | _ | 0 | I |
| C12 1733 | + | + + | - | - | - | + | - | + | 0 | ND |
| C15 1962 | + | + | - | - | - | + | - | + | - | ND |
| C15 7785 | + | + | + | + | GGCCC | + + | + + | + | 0 | F |
| P16 5242 | + | + | + | + | CV | + | - | + | 6 | I |
| C18 113 | + | +/- | - | - | - | + | - | +/- | 0 | ND |

EC: Eosinophilic cells; PSP: pseudopapillary formation; LGT C: light cells; AP: apoptosis; DGC: degenerative changes HE: hemorrhage ; F: fibrosis; A: atypia (multiple nuclei, increased volume, nucleoli); M: mitosis (10 / large magnification field); C: capsule; AEG: eosinophilic granules; CN:

coagulation necrosis; C: calcification; GGCCC: granuloma with giant cells and cholesterol crystals; GT: granulation tissue; CV: cellular vacuolation; FI: fibrous invasion; F: fibrosis; I: invasion; ND: Not determined; + positive; negative -

## RESULTS

The macroscopic examination revealed rounded or oval masses, measuring between 2.5 x 2 x 2 cm and 10 x 7 x 6 cm, of a firm and elastic consistency, apparently encapsulated, three located in the head of the pancreas and two in the middle body/tail region of the pancreas. In the sections, it was possible to see clear and regular borders and whitish or yellowish surfaces, with solid areas located mainly in the periphery of the tumor, and areas sometimes grainy, others soft, associated with the hemorrhagic areas (Fig. 1A).

The histopathological examination of the slides stained with hematoxylin and eosin showed isolated neoplasms of the pancreatic parenchyma by fibrous pseudocapsule (Fig. 1B), which was permeated in three cases, but not crossed by neoplastic cells. They were polyedric, little cohesive, with eosinophilic (Fig. 2A) or light (Fig. 2B) cytoplasm, forming cell masses permeated by a delicate connective-vascular stroma. The nuclei were rounded or oval, with regular contours or slightly ribbed, or even with mild anisokaryosis. Cells with hyperchromatic nuclei, sometimes multiple, were present, focally, in one of the cases. The number of mitoses ranged from zero (five cases) to six (one case) in ten fields of large magnification. The neoplastic cells were frequently positioned perpendicularly around the axis, configuring pseudopapillary formations, on which occasion the cytoplasm appeared to be more elongated, and the nuclei were

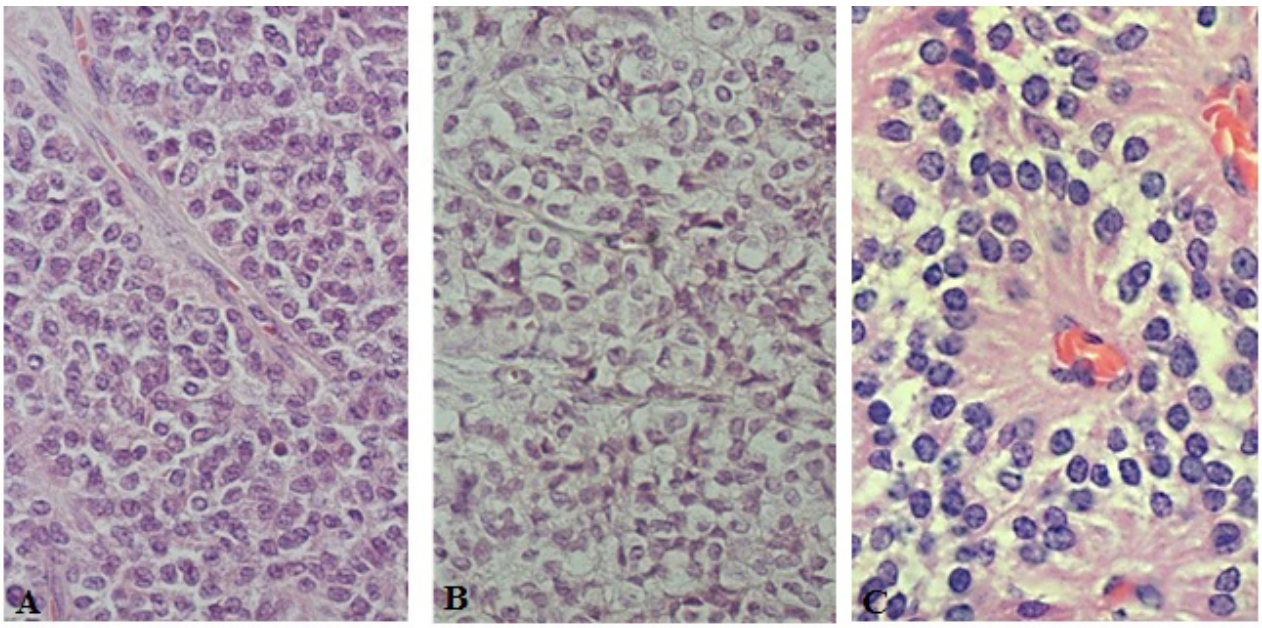


**FIGURE 2.**

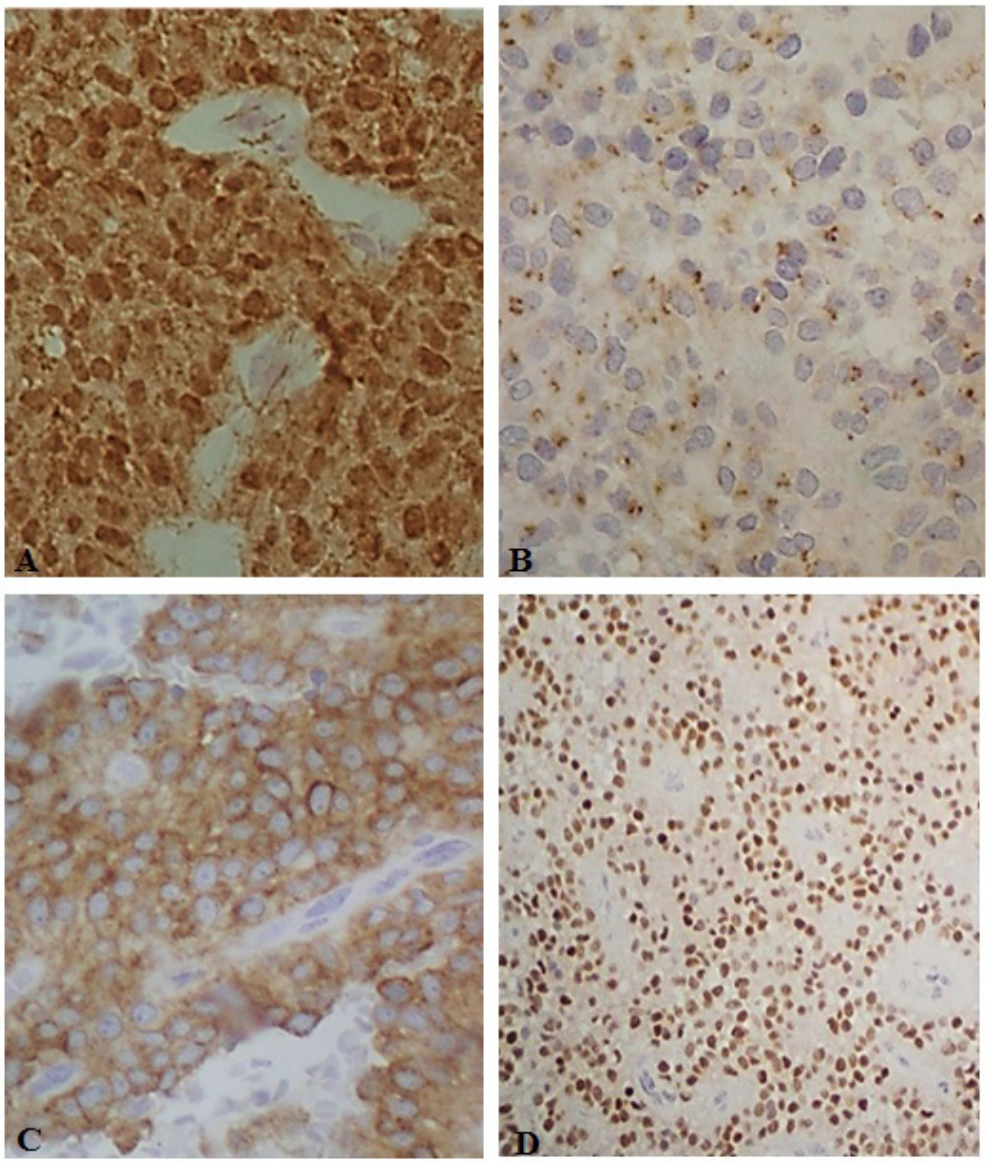


**FIGURE 3.**

**TABLE 3.** IMMUNOHISTOCHEMISTRY

| Biopsy No | B-catenin | CD99 | CD10 | CD56 | Progesterone receiver | Chromo granin | Synapto-physin | Ecaderin | Ki-67 | OBS |
|---|---|---|---|---|---|---|---|---|---|---|
| B2116-97 | NP | POS | POS | NP | POS | NEG | NEG | NP | <2% | 1 bl |
| P00 3249 | POS | POS Focal | POS | NEG | POS | NEG | NEG | NEG | <2% | 1 bl |
| P11 1978 | POS | POS 1 bl NEG 2 bl | POS | POS | POS | POS focal 1 bl; NEG 2 bl | NEG | NEG | <2% | 3 bl |
| C12 1733 | NP | POS | POS | NP | POS | NEG | NEG | NP | <2% | 1 bl |
| C15 1962 | POS | POS | POS | NP | POS | NEG | NEG | NP | <2% | 2 bl |
| C15 7785 (C15-1962) | POS | POS | POS 1 bl; NEG 1 bl | POS 1 bl; NEG 1 bl | POS | NEG | POS focal 1 bl | NEG | <2% | 2 bl |
| P16 5242 | POS | POS 1 bl NEG 2 bl | POS | POS | POS | NEG | NEG | NEG | 8% | 3 bl |
| C18 113 | POS | NEG | POS | POS | POS | NEG | NEG | NEG | <2% | 1 bl |

POS: positive; NEG: negative; NP: the exam was not performed; bl: block.

located in the apical edge of the cell (Fig. 2C). Areas formed by granulation tissue and multinucleated giant cells containing cholesterol crystals were observed in two cases. Hemorrhagic foci and cell degeneration were observed in all cases, and fibrosis in four cases, with varying intensity. Eosinophilic granules, intra or extracellular, were observed in two cases and were positive to staining by PAS in one case and negative in another.

There were no significant histological changes in the pancreatic parenchyma adjacent to the neoplasms.

The immunohistochemical assay confirmed the diagnosis of SPNP by the positivity of the neoplastic cells, in all cases studied, to the anti-beta-catenin antibodies in nuclear and cytoplasmic locations (Fig. 3A), the anti-CD99 of cytoplasmic granular pattern Fig.3B), the anti-CD-10 in cytoplasmic location Fig. 3C), the anti-progesterone receptor in nuclear location (Fig. 3D), and by the negativity to anti-E-cadherin, which are considered the main markers of this neoplasm. In addition, the negativity, in all cases, to the anti-chromogranin A and anti-synaptophysin antibodies favored the differential diagnosis with the neuroendocrine neoplasms. The proliferative index assessed by the nuclear reaction in the neoplastic cells, with the anti-KI67 antibody, was lower than 2% in three cases and 8% in one case. This also presented a high mitotic index (six mitosis/ten fields of large magnification) and is in regular outpatient monitoring since 2016, so far, uneventfully (Table 3 and Figure 3).

## CONCLUSION

Solid pseudopapillary neoplasms of the pancreas have a heterogeneous pattern regarding their macroscopic, microscopic, immunophenotypic, and molecular aspects, as evidenced both in the bibliographical review, as in the cases studied. The histopathological diagnosis is guided by the presence of solid and pseudopapillary areas; however, the immunohistochemistry assists in the differential diagnosis with other pancreatic neoplasms, mainly by the aberrant nuclear expression of beta-catenin, associated to the lack of membrane expression of E-cadherin, the typical perinuclear granular marking of CD99, and CD10 positivity. Molecular biology is still poorly understood, although many studies on the subject have been published. Although rare and having, in most patients, good prognosis and excellent response to surgical treatment, it is a neoplasia that, due to its enigmatic cell origin and its morphological and molecular heterogeneity, encourages the search for a better understanding of its biology.

### Author Contribution

Vera Lucia Chagas wrote the manuscript, and all authors reviewed it and made contributions.

## RESUMO

OBJETIVO: *Fazer revisão da literatura e do diagnóstico histopatológico convencional de rotina e de imuno-histoquímica dos casos diagnosticados da neoplasia sólida pseudopapilar do pâncreas (NSPP).*

MÉTODOS: *A revisão da literatura foi feita utilizando as bases de dados PubMed e Google Scholar, por meio do histórico, aspectos clínicos e métodos de diagnóstico da NSPP. A revisão dos casos de NSPP diagnosticados no Hospital Universitário Clementino Fraga Filho da UFRJ foi feita no período de 1997 a 2018.*

RESULTADOS: *A heterogeneidade fenotípica intratumoral da NSPP foi evidenciada nos casos estudados, levando-se em conta os padrões macroscópicos, microscópicos e imuno-histológicos.*

CONCLUSÕES: *O conjunto de resultados evidencia a importância do exame de vários fragmentos obtidos de regiões distintas das neoplasias, uma vez que nem todos eles apresentam as mesmas alterações moleculares.*


PALAVRAS-CHAVE: *Pâncreas. Neoplasia sólida pseudopapilar.*

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COMMENTARY

# Comments "Solid pseudopapillary neoplasia of the pancreas: a review"

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Solid pseudopapillary neoplasm of the pancreas is a type of tumor that, since its initial description, in 1959, by Virginia Kneeland Frantz, caused a series of controversies regarding its biological behavior. Several names have been used to describe this singular disease in publications, among them, Frantz tumor, as a tribute to the eminent American pathologist. This fact, associated with the rarity of the disease, was one of the contributing factors for its true epidemiology to remain unknown in past decades.

It was, without a doubt, the progress of imaging methods associated with a better knowledge of histopathological techniques and, fundamentally, immunohistochemistry that made its diagnosis more frequent in recent years. Although its clinical presentation is not pathognomonic, it is usually a result of the neoplasia mass effect and, not rarely, its diagnosis is incidental. It affects mostly young women, although it has been described in different age groups; however, it rarely occurs in males.

There does not seem to be any controversy regarding the treatment of choice, which consists of surgical resection with margins. More recently, the need for lymphadenectomy has been discussed due to the description of ganglionar metastases in approximately 15% of cases.

Despite the advances regarding the knowledge about this neoplasia[4], which is mostly from retrospective studies of series and case reports due to the low incidence of the condition, there are still gaps in respect to its cellular origin, etiopathogenesis, and, fundamentally, about the true biological behavior of the tumor.

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# Comments": "Application of low-dose CT screening can reduce cancer mortality"

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We read the study by Xue Tang[1] and colleagues with great interest, in which they identified that low-dose CT (LDCT) screening for lung cancer is effective in reducing the mortality rate of lung cancer in high-risk smokers. This information is of great significance in the prevention and early diagnosis of lung cancer, providing strong evidence for formulating health policies. However, we believe there are still some deficits, and that stronger evidence should be included for drawing the cause and effect conclusion between LDCT screening and decrease of lung cancer mortality.

To begin with, the authors should take into consideration many other protective effects following LDCT screening. For example, smoking cessation is a well-known protective factor for reducing lung cancer mortality[2]. Changes in lifestyle after the lung cancer diagnosis, such as smoking cessation, can also result in the decrease of lung cancer mortality. Furthermore, early treatment and intervention following early diagnosis by LDCT screening can also be a strong protective factor[3]. Therefore, more factors should be considered when exploring the cause and effect relationship between LDCT screening and low-dose cancer mortality.

Furthermore, population bias should be appropriately excluded. Candidates included in LDCT screening tend to receive more emphasis on their health condition. Therefore, other measures such as regular serological examination might be performed, which can also elevate the possibility of lung cancer diagnosis and decrease the mortality rate of lung cancer[4]. Therefore, population-based studies should be included in future meta-analyses.

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