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Vários anos se passaram. Assumi a Clinical & Biomedical Research sucedendo ao editor chefe Alexandre Zavascki. Encaramos a responsabilidade de qualificar a CBR. Estávamos conscientes que a CBR precisa ter visibilidade e, principalmente, ter um atestado de qualidade. Promovemos uma reestruturação da CBR, qualificamos a revisão por pares e reformulamos o corpo editorial. A produção científica brasileira pode sustentar a ambição de termos uma revista forte. É visível o progresso que obtivemos. Hoje, podemos vislumbrar novas indexações. A CBR não se acomodou! Progressivamente foi reformulada em busca dos padrões internacionais. A CBR precisa ser ambiciosa! Nada disso, entretanto, teria sido possível sem a ajuda de muitos. A primeira pessoa a agradecer é o Prof. Eduardo Passos que me convidou quando coordenava o então GPPG. O Eduardo sempre me apoiou e esteve atento à CBR. Outros colaboradores foram fundamentais como o José Goldin, Francisco Veronese e o Afonso Luis Barth e a secretária Rosa que se aposentou. Em plena pandemia, o Michael Andrades se juntou a nós e fez um trabalho incrível de atualização das políticas editoriais da CBR. É assim que vamos passar o bastão. O novo editor é o Daniel Umpierre. O Daniel tem tudo para ser melhor que eu, mas continuo ao lado da CBR como um dos editores associados. Vamos dar as boas vindas ao Daniel e a quem quiser se juntar ao nosso time.

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BDNF as a potential biomarker for the fibromyalgia-like model in male Wistar rats

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ABSTRACT

Introduction: Fibromyalgia syndrome is characterized by central sensitization, with imbalance between the descending pain inhibition pathways and the ascending pain signaling pathways, including changes in the serotonergic, dopaminergic and catecholaminergic circuits. The aim was to evaluate the nociceptive response, depression-like behavior, and central and peripheral biomarkers levels (BDNF and TNF-α) in male adult Wistar rats submitted to a fibromyalgia-like model induced by reserpine.

Methods: Sixteen male adult Wistar rats were allocated by weight in control and fibromyalgia groups. Mechanical and thermal hyperalgesia were evaluated by Von Frey test and Hot Plate test respectively, and depression-like behavior was evaluated by Forced Swim test. Also BDNF and TNF-α were measured in serum and central structures.

Results: Rats in the fibromyalgia group presented a lower thermal and mechanical threshold, and an increased immobility time. also reduced serum BDNF levels, without changes in TNF-α levels. There was a positive correlation between mechanical and thermal nociceptive threshold and serum BDNF levels, and a negative correlation between thermal nociceptive threshold and spinal cord BDNF levels. Also, there was a negative correlation between immobility time and serum BDNF levels.

Conclusion: In summary, this study provides confirmation that the fibromyalgia-like model induced by reserpine effectively replicates the symptoms of fibromyalgia. Additionally, it provides evidence supporting the involvement of brain-derived neurotrophic factor (BDNF) in the development of this pain model.

Keywords: *Fibromyalgia; Rats, BDNF; TNF-α*

INTRODUCTION

Fibromyalgia syndrome is characterized by chronic widespread pain, fatigue, non-restorative sleep, and cognitive symptoms[1], afflicting mainly women and affecting nearly 2% of the world population. The main pathophysiological phenomenon of fibromyalgia is the central sensitization, characterized by a limitation of descending pain inhibition pathways and by the improvement of the ascending pain signaling pathways[2]. Several pathophysiological hypotheses have been proposed to elucidate this syndrome, which the most accepted considers that there is an imbalance between nociception and physiological pain control[3]. According to this hypothesis, there is a global decrease in inhibitory pathways related to the pain, thereby allowing low-intensity or non-nociceptive stimuli to be processed in pre-cortical and cortical structures involved in the affective and cognitive pain process, resulting in an increase of painful perception[4]. Therefore, therapeutic strategies aim to modulate neuroplasticity processes in the pain neuromatrix (multiple brain areas implied in affective, cognitive, and evaluative responses to pain).

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Animal models of disease allow us to evaluate hypotheses through experiments that cannot be tested on humans. Due to the serotonergic, dopaminergic and catecholaminergic described circuit changes, in fibromyalgia patients, a model of this disease has been proposed in rats, using the drug reserpine[5]. The repeated injection of reserpine can induce depletion of biogenic amines in rats, inducing generalized allodynia, a finding that is characteristic of this disease. In this way, this model of the disease offers a plausible neurobiological substrate, with a consistent disease behavioral correlation which thus offers subsidies to study the combination of pharmacological and non-pharmacological intervention, their behavioral, neuroanatomical, and neurobiological effects.

In face of this, the aim was to evaluate the nociceptive response, depression-like behavior, and central and peripheral biomarkers levels (BDNF and TNF-α) in male adult Wistar rats submitted to a fibromyalgia-like model induced by reserpine.

MATERIALS AND METHODS

Animals

Sixteen male Wistar rats [55 – 65 days old (≃ 250g) at the beginning of treatment were used, which was provided by the Animal Reproduction and Experimentation Center (CREAL) through the Institute of Basic Health Sciences (ICBS) of Federal University of Rio Grande do Sul (UFRGS). Rats were assigned or weight-matched (in grams) and housed in groups of 2 to 3 rats per polypropylene cage (49 cm × 34 cm × 16 cm). Rats were kept at a 12-hour light/dark standard cycle (lights on at 7am and lights off at 7pm), in a controlled temperature environment (22 ± 2° C) having free access to water and to Nuvital® feed.

All experiments and procedures were approved by the Institutional Committee for Animal Care and Use (GPPG-HCPA protocol Nº 2015-0272) and according to the Guide for the Care and Use of Laboratory Animals 8th ed. 2011 and law 11.794 (Brazil). The experimental protocol complied with the ethical and methodological standards of the ARRIVE guidelines[6]. The sample size was calculated to detect the statistical significance between means considering an alpha = 0.05 and power of 90%[7].

Experimental Design

Rats were assigned or weight-matched and divided into 2 experimental groups: Control (vehicle) and fibromyalgia (reserpine). The mechanical hyperalgesia threshold was evaluated by the electronic von Frey test before the administration of reserpine and five days after the last dose of the drug. Also, forced swim and hot plate tests were performed five days after the last dose of the drug (Figure 1).

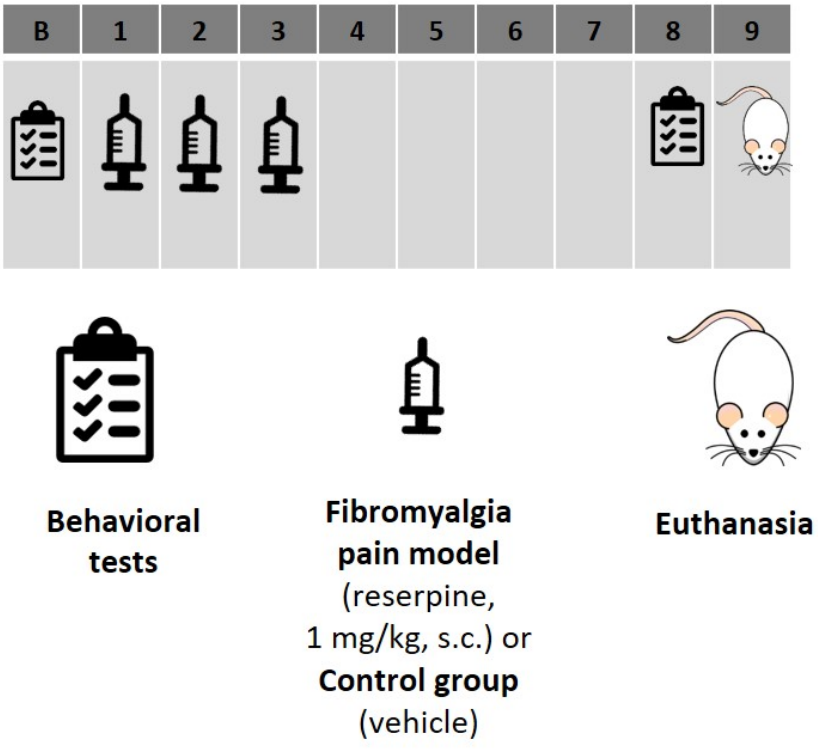

Figure 1: Experimental design.

Fibromyalgia Model

To induce a fibromyalgia-like model, the rats received reserpine (1 mg/kg, s.c.) diluted in 0.5% acid acetic glacial (vehicle) for three consecutive days. In this way, there were two experimental groups, one received vehicle and the other, reserpine. Reserpine can induce depletion of biogenic amines in rats, inducing generalized hyperalgesia, a finding that is characteristic of the disease[7]. Five days after the last reserpine administration (8th day), the rats were assessed for behavioral tests (von Frey, hot plate and forced swim). For weight maintenance of the rats subjected to the reserpine model, it was needed to add sunflower seed, because of significant weight loss, since 20% means in euthanasia of rats. This sunflower seed was added only during 3 days from 8th to 10th day after first reserpine injection. The amount added was 3 to 5 g per cage per day.

Von Frey Test

Mechanical hyperalgesia was assessed by the electronic von Frey test[8]. First, rats were allowed to acclimate in acrylic boxes on a wire metal grid for 15 minutes 24h before testing to avoid stress-induced novelty. Then, in the next day rats were allowed to the boxes again, and after 5 minutes a plastic tip was perpendicularly probed under the animal's right rear paw. Flick, biting or shaking responses were taken as nociceptive measures and recorded in grams (g) throughout the mean of 3 trials.

Hot Plate Test

For thermal hyperalgesia, the rats were also acclimated 24h before testing for allowing them to explore the apparatus for 5 minutes in the switched off mode. On day testing the temperature was set up to 55° C. After that, rats were lowered on a warmed surface circumvented by a transparent polypropylene funnel. The time between the placement, and the first aversive response to thermal noxious stimulus understood as paw shaking or biting was timed in a single trial and expressed in seconds (s). A 20 second cutoff was established to avoid tissue damage[9].

Forced Swim Test

This test was performed to assess depressive-like behavior. The animals were placed into a round glass tank (dimensions 40 cm x 40 cm x 52 cm), the tank was filled with water (22-25° C) until a depth of 35 cm, in such a way that the tail of the rats could not touch the bottom of the tank. The animals were placed into water for five minutes, the immobility time of the rat was recorded in seconds, considering the total of immobility and/or movements to keep the head out of the water with no intention of escaping. After the test, the rats were dried with cotton pads and a hairdryer[10]. The tests were filmed and analyzed by two trained researchers, with the goal of subsequently performing a double check of obtained results to guarantee their reliability and was carried out five days after the last dose of reserpine.

Tissue collection

Rats were killed by decapitation 24 hours after the last behavioral evaluation; and the cerebral cortex, spinal cord, hippocampus, and brainstem were harvested and immediately stored in the - 80° C freezer. Also, the serum was collected and stored in the - 80° C freezer for further analysis.

Biochemical assays

TNF-α and BDNF levels were determined by enzyme-linked immunosorbent assay (ELISA) using monoclonal antibodies for each cytokine and neurotrophin (R&D Systems, Minneapolis, United States) using the manufacturer's protocol. Optical density was measured using an ELISA reader at a wavelength of 450 nm. Total protein was measured by the Bradford method using bovine serum albumin as the standard[11]. Data were expressed in picograms per milligram (pg/mg) of protein.

Statistical analysis

For behavioral and biochemical data, a t test for independent samples (Student t test) was used by comparing the control group and fibromyalgia-like model. Spearman's correlation was made between behavioral tests and BDNF levels. A $P < 0.05$ was taken as significant. All data are expressed as mean ± standard error of the mean (S.E.M.) and were run in the SPSS 26.0 statistical program.

RESULTS

Behavioral measures

Regarding mechanical hyperalgesia, it was found to be an interaction between group vs time (repeated measures ANOVA, $P < 0.001$, $F^{1,12} = 0.660$). At baseline, there was no difference between groups upon the mechanical hyperalgesia (Figure 2 Panel A). However, on the 8th day, after the reserpine-induced fibromyalgia-like model, there was a difference between groups; rats that received reserpine displayed a decrease in the mechanical nociceptive threshold compared to those that received vehicle (Figure 2, Panel A, $n = 7 – 8$ per group).

Regarding thermal sensitivity, we found differences between groups by showing that reserpine-induced

fibromyalgia-like model group displayed a marked thermal hyperalgesia compared to the vehicle group five days after the last injection (independent Student t test, P = 0.033; Figure 2, Panel B; n = 8 per group).

Concerning the forced swimming test, we also noted that a reserpine-induced fibromyalgia model triggered an increased immobility time compared to vehicle groups (independent Student t test, P < 0.001, Figure 2, Panel C, n = 5 per group).

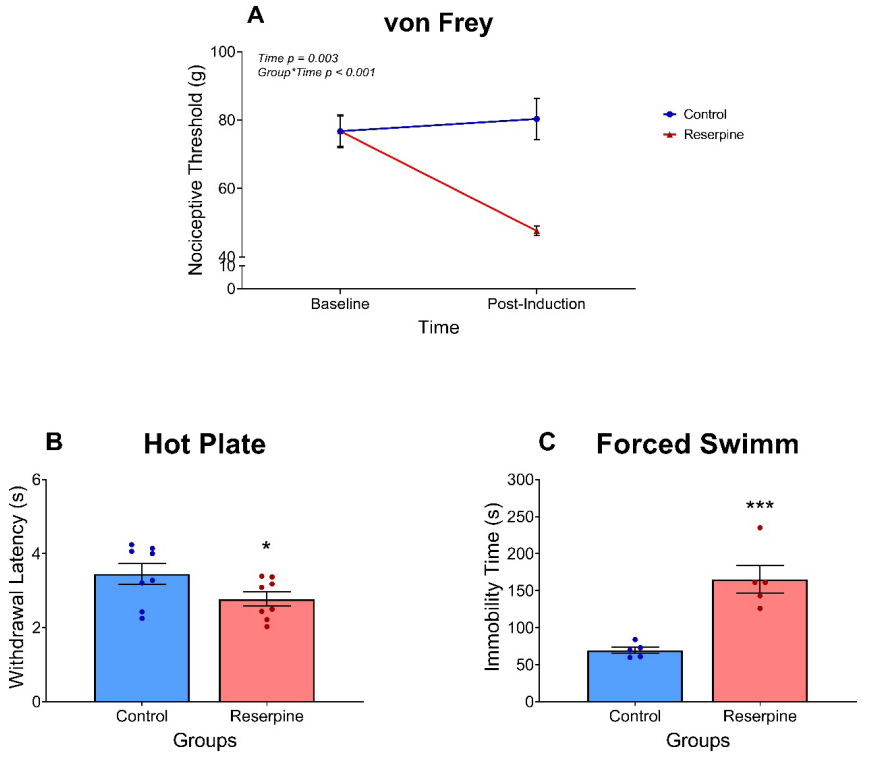

Figure 2: Behavioral assessments.

A: Mechanical hyperalgesia threshold assessed in the Von Frey test at baseline and after induction of fibromyalgia model (8th day). *Significant difference from control group (repeated measures ANOVA, P < 0.001). The vertical lines indicate the mean ± S.E.M of paw withdrawal threshold in grams (g); B: Thermal hyperalgesia assessed by the Hot Plate test. The vertical lines indicate the mean ± S.E.M of paw withdrawal threshold in seconds (s) *Significant difference from control group (Student t test, P = 0.033); C: Immobility time assessed by the Forced Swim test. The vertical lines indicate the mean ± S.E.M of immobility time in seconds (s). *Significant difference from control group (Student t test, P < 0.001).

Biochemical Analysis

Regarding cerebral cortex, brainstem, and spinal cord BDNF levels, we found no statistical difference between groups (independent Student t test, P = 0.453, P = 0.507, P = 0.576, respectively; n = 7 – 8 per group, Table 1). However, the serum BDNF levels were decreased in rats subjected to reserpine-induced fibromyalgia rat model in relation to the control group (independent Student t test; P < 0.001; n = 7 – 8 per group, Table 1).

Regarding TNF-α, no significant differences were found in all evaluated structures (hippocampus, brainstem, cerebral cortex, and serum) (independent Student t test, P = 0.213, P = 0.0.262, P = 0.120, P = 0.459, respectively; n = 7 – 8 per group, Table 1).

Table 1: Biomarkers profile after reserpine-induced fibromyalgia rat model in serum and central nervous system structures (brainstem, cerebral cortex, spinal cord and hippocampus). Data expressed as mean ± SEM (pg/mg of protein) of biomarkers levels in the central nervous structures and serum of rats.

Biomarker	Structure	Control group	Fibromyalgia group	P value
BDNF (pg/mg of protein)	Brainstem	3.23±0.21	3.44±0.21	0.507
	Cerebral Cortex	0.61±0.15	0.46±0.12	0.453
	Spinal Cord	3.11±0.44	2.59±0.75	0.576
	Serum	0.60±0.01	0.45±0.02*	<0.001

Continues...

Table 1: Continuation.

Biomarker	Structure	Control group	Fibromyalgia group	P value
TNF-a (pg/mg of protein)	Brainstem	2.15±0.44	3.29±0.84	0.262
	Cerebral Cortex	1.87±0.49	0.88±0.29	0.120
	Hippocampus	2.17±1.05	0.71±0.13	0.213
	Serum	10.86±2.59	13.29±1.79	0.459

* independent Student t test.

Behavioral tests and BDNF: correlation Analysis

We have investigated the correlation between behavioral tests (mechanical and thermal threshold and immobility time) with BDNF levels (brainstem, cerebral cortex, spinal cord, and serum) in Figure 3. It was found a positive correlation between mechanical nociceptive threshold and serum BDNF levels (Spearman's correlation, P < 0.001, r = 0.841); however, there were no correlation between mechanical nociceptive threshold and brainstem BDNF levels (P = 0.474), and spinal cord BDNF levels (P = 0.201) and cerebral cortex BDNF levels (P = 0.748).

Also, a negative correlation was found between thermal nociceptive threshold and spinal cord BDNF levels (Spearman's correlation, P=0.031, r = - 0.558) and a positive correlation between serum BDNF levels was found (P = 0.034, r = 0.532); however there were no correlation between thermal nociceptive threshold and brainstem BDNF levels (P = 0.242), and cerebral cortex BDNF levels (P = 0.879).

In addition, a negative correlation was found between immobility time and serum BDNF levels (Spearman's correlation, P = 0.006, r = - 0.796); however there were no correlation between immobility time and spinal cord BDNF levels (P = 0.700), brainstem BDNF levels (P = 0.455) and cerebral cortex BDNF levels (P = 0.815).

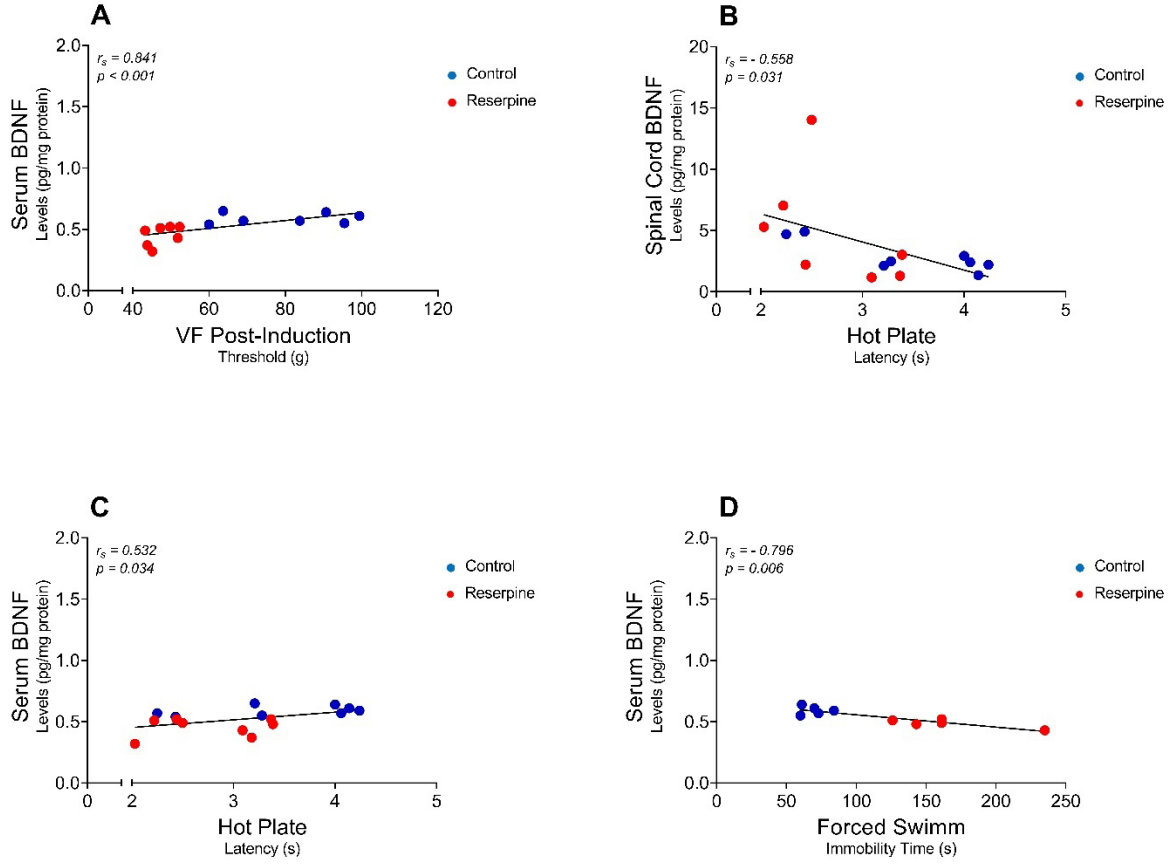

Figure 3: Correlation analysis between behavioral measurements and BDNF levels.

A: Spearman's correlation between mechanical nociceptive threshold and serum BDNF levels; B: Spearman's correlation between thermal nociceptive threshold and spinal cord BDNF levels; C: Spearman's correlation between thermal nociceptive threshold and serum BDNF levels.

DISCUSSION

The results of the current study demonstrated that rats submitted to the fibromyalgia model presented mechanical and thermal hyperalgesia, an increase in immobility time and a reduced serum BDNF levels. In addition, there was a positive correlation between mechanical and thermal nociceptive threshold and serum BDNF levels. On the other hand, there is a negative correlation between thermal nociceptive threshold and spinal cord BDNF levels. Also, there was a negative correlation between immobility time and serum BDNF levels. Altogether, these results outline that reserpine-induced fibromyalgia-like models can unleash behavioral phenotypes related to chronic pain, probably with participation of the peripheral BDNF levels.

It is important to note that on the third day of reserpine administration for fibromyalgia model induction, the rats showed an eye compression, a vocalization at the manipulation moment, an arched posture, a weight loss and a prostration behaviors that indicate the presence of pain[12]. According to the Grimace scale for rats (orbital tightening, nose/cheek flattening, ear changes and whisker change)[13], in the current study, the rats presented an indication of severe pain, and loss of weight (data not shown). Altogether, our findings showed that rats in the fibromyalgia model displayed a hyper nociceptive behavior, such as thermal and mechanical hyperalgesia, both linked to central sensitization[14,15].

Additionally, in the current study, the lower thermal threshold was characterized by presenting only ocular compression and vocalization for thermal sensitivity signaling, and not presenting the expected response which was the “tap dance” movement and the licking of the feet (data not shown)[9]. Previous study showed that monoamines (serotonin, norepinephrine, and dopamine) are reduced in the spinal cord in rats subjected to an animal model of fibromyalgia[5], which may contribute to lower thermal threshold. However, it is possible to suggest that the fibromyalgia rats present alterations in the supraspinal pain process, since the hot plate test involves complex supraspinal sensory integration[16].

A clinical diagnosis from the patient’s complaints related to several complicated spots throughout the body, such as pain, aches, palpitations, and often psychopathological changes, such as anxiety and depression[17]. Also, evidence suggests that patients diagnosed with fibromyalgia have reduced serum levels of BDNF, corroborating the worsening of the psychopathologies involved in this condition[18]. In agreement, this current study showed that the fibromyalgia model induced a reduction in serum BDNF levels of rats. It is important to note that the scientific literature showed that BDNF is an important biomarker in different types of pain, including fibromyalgia[19], also in the peripheral and central nervous structures. Moreover, it is suggested the role of cytokines in pathogenesis of fibromyalgia; an increased plasma TNF-α, IL-10 and IL-8 were observed in fibromyalgia patients; a reduced level of anti-inflammatory cytokine IL-5, but a normal level in IL-10[20]. However, we did not observe any effect on TNF-α central and peripheral levels.

We have found a negative correlation between thermal nociceptive threshold and BDNF spinal cord levels. Also, there was a positive correlation between mechanical and thermal nociceptive threshold and BDNF serum levels. Our results suggest a different role of BDNF in central and peripheral levels. Confirming our hypothesis, a previous preclinical study showed that a single intrathecal injection of BDNF was able to decrease the nociceptive threshold in naive rats, for at least a 42-day period[21]. Another study showed decreased development of allodynia and hyperalgesia in the post-injury period in BDNF-deficient subjects[22]. This effect of BDNF can be seen in both the peripheral sensory neuron and the spinal cord, where BDNF is released by microglia[22]. Corroborating these data, a clinical study showed that the concentration of plasma BDNF levels was positively associated with the heat pain threshold and numeric rating scale of pain[23]. In this way, the possible BDNF upregulation in the dorsal root ganglion (DRG) and spinal cord, which contributes to chronic pain hypersensitivity, may indicate that blocking BDNF in sensory neurons provides a new target for the development of novel drugs[24].

Furthermore, depressive-like behavior was assessed, the fibromyalgia rats presented a significant increase in immobility time, indicating the depressive-like behavior. Previous studies have used this model also to evaluate antidepressant drugs[25,26]. Thus, the depletion of monoamines resulting from the administration of reserpine may be reproducing, in addition to fibromyalgia, comorbidities attributed to this condition, such as depression. It is known that the pathophysiology of depression is complex, but there is an involvement with deficiency of monoaminergic neurotransmitters, specifically norepinephrine (NE) and serotonin (5-HT)[27]. In addition, a negative correlation was found between immobility time and BDNF serum levels. This result corroborated another study that showed a reduction in this neurotrophin induced by a fibromyalgia-like model conducted by intermittent cold stress with slight modification[28]; despite this, we did not observe change in the central BDNF levels, at least in the structures evaluated. In agreement with our data, a previous study showed a significant negative correlation between BDNF expression levels with immobility time[29].

Indeed, neurotrophic factors play a central role in the synaptic plasticity, and they may be involved in the maladaptive neuroplasticity in depression.

In conclusion, this study provides confirmation that the fibromyalgia-like model induced by reserpine effectively replicates the symptoms of fibromyalgia. Additionally, it provides evidence supporting the involvement of BDNF in the development of this pain model. Rats with fibromyalgia exhibited a reduction in peripheral levels of BDNF. Furthermore, there was a positive correlation observed between the reduced levels of BDNF and both the thermal and mechanical nociceptive threshold. On the other hand, there was a negative association between the central levels of BDNF and the thermal nociceptive threshold. The statement above emphasizes the importance of identifying biomarkers for fibromyalgia that contribute to the differentiation of diagnosis and facilitation of therapy. Additionally, this will also provide a substantial contribution to future research endeavors aimed at assessing the efficacy of both pharmaceutical and non-pharmacological therapeutic methods.

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Authors' contributions

VAS, LFM, CLO, HRM, JADS, AS and ILST have made substantial contributions to conception and design, or acquisition of data, or analysis and interpretation of data. VAS, LFM, DJS and ILST have been involved in drafting the manuscript or revising it critically for important intellectual content. All authors have given final approval of the version to be published.

REFERENCES

1. Ceca D, Pablos A, Elvira L, López-Hernández L, Ortega AL. Effectiveness of a self-myofascial conditioning programme on pain, depression, anxiety and sleep quality in people with Fibromyalgia. *Cuad Psicol del Deport*. 2020;20(1):147-65.
2. Poluha RL, Grossmann E. Does pregabalin improve sleep disorders in fibromyalgia? TT – A pregabalina melhora os distúrbios do sono na fibromialgia? *BrJP*. 2018;1(2).
3. Yunus MB. Fibromyalgia and overlapping disorders: the unifying concept of central sensitivity syndromes. *Semin Arthritis Rheum*. 2007;36(6):339-56.
4. Burgmer M, Pogatzki-Zahn E, Gaubitz M, Wessoleck E, Heuft G, Pfleiderer B. Altered brain activity during pain processing in fibromyalgia. *Neuroimage*. 2009;44(2):502-8.
5. Nagakura Y, Oe T, Aoki T, Matsuoka N. Biogenic amine depletion causes chronic muscular pain and tactile allodynia accompanied by depression: a putative animal model of fibromyalgia. *Pain*. 2009;146(1-2):26-33.
6. Kilkenny C, Browne WJ, Cuthill IC, Emerson M, Altman DG. Improving bioscience research reporting: The arrive guidelines for reporting animal research. *PloS Biol*. 2010;8(6):e1000412.
7. Nagakura Y, Takahashi M, Noto T, Sekizawa T, Oe T, Yoshimi E, et al. Different pathophysiology underlying animal models of fibromyalgia and neuropathic pain: Comparison of reserpine-induced myalgia and chronic constriction injury rats. *Behav Brain Res*. 2012;226(1):242-9.
8. Cioato SG, Medeiros LF, Marques Filho PR, Vercelino R, de Souza A, Scarabelot VL, et al. Long-lasting effect of transcranial direct current stimulation in the reversal of hyperalgesia and cytokine alterations induced by the neuropathic pain model. *Brain Stimul*. 2016;9(2):209-17.
9. Woolfe G, Macdonald AD. The evaluation of the analgesic action of pethidine hydrochloride (Demerol*). J Pharmacol Exp Ther*. 1944;80(3):300-7.
10. Moreira SFS, Nunes EA, Kuo J, de Macedo IC, Muchale A, de Oliveira C, et al. Hypoestrogenism alters mood: Ketamine reverses depressive-like behavior induced by ovariectomy in rats. *Pharmacol Rep*. 2016;68(1):109-15.
11. Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein using the principle of protein–dye binding. *Anal Biochem*. 1976;72:248-54.
12. Neves SMP, Mancini Filho J, de Menezes EW. *Manual de cuidados e procedimentos com animais de laboratório do Biotério de Produção e Experimentação da FCF-IQ/USP*. São Paulo: FCF-IQ/USP; 2013.
13. Sotocinal SG, Sorge RE, Zaloum A, Tuttle AH, Martin LJ, Wieskopf JS, et al. The Rat Grimace Scale: a partially automated method for quantifying pain in the laboratory rat via facial expressions. *Mol Pain*. 2011;7:55.
14. Price DD, Staud R, Robinson ME, Mauderli AP, Cannon R, Vierck CJ. Enhanced temporal summation of second pain and its central modulation in fibromyalgia patients. *Pain*. 2002;99(1-2):49-59.
15. Staud R, Rodriguez ME. Mechanisms of disease: pain in fibromyalgia syndrome. *Nat Clin Pract Rheumatol*. 2006;2(2):90-8.
16. Kiso T, Moriyama A, Furutani M, Matsuda R, Funatsu Y. Effects of pregabalin and duloxetine on neurotransmitters in the dorsal horn of the spinal cord in a rat model of fibromyalgia. *Eur J Pharmacol*. 2018;827:117-24.
17. Alves RC, Nepomuceno VR, Marson PG, Bartholomeu Neto J, Silveira JM, Silveira JM, et al. Aspectos epidemiológicos e diagnóstico da

fibromialgia na região Norte do Brasil. *Res Soc Dev*. 2022;11(4): e3511427704.

18. Merino AS, Simon D. Fatores genéticos associados à fibromialgia: uma revisão narrativa. *Res Soc Dev*. 2022;11(3): e11211326421.

19. Siracusa R, Di Paola R, Cuzzocrea S, Impellizzeri D. Fibromyalgia: pathogenesis, mechanisms, diagnosis and treatment options update. *Int J Mol Sci*. 2021;22(8):3891.

20. Singh L, Kaur A, Bhatti MS, Bhatti R. Possible molecular mediators involved and mechanistic insight into fibromyalgia and associated co-morbidities. *Neurochem Res*. 2019;44(7):1517-32.

21. Constandil L, Aguilera R, Goich M, Hernández A, Alvarez P, Infante C, et al. Involvement of spinal cord BDNF in the generation and maintenance of chronic neuropathic pain in rats. *Brain Res Bull*. 2011;86(5-6):454-9.

22. Coull JAM, Beggs S, Boudreau D, Boivin D, Tsuda M, Inoue K, et al. BDNF from microglia causes the shift in neuronal anion gradient underlying neuropathic pain. *Nature*. 2005;438(7070):1017-21.

23. Sorkpor SK, Galle K, Teixeira AL, Colpo GD, Ahn B, Jackson N, et al. The relationship between plasma BDNF and pain in older adults with knee osteoarthritis. *Biol Res Nurs*. 2021;23(4):629-36.

24. Obata K, Noguchi K. BDNF in sensory neurons and chronic pain. *Neurosci Res*. 2006;55(1):1-10.

25. Gao ZY, Yang P, Huang QJ, Xu HY. The influence of dizocilpine on the reserpine-induced behavioral and neurobiological changes in rats. *Neurosci Lett*. 2016;614:89-94.

26. Kim YR, Park BK, Seo CS, Kim NS, Lee MY. Antidepressant and anxiolytic-like effects of the stem bark extract of fraxinus rhynchophylla hance and its components in a mouse model of depressive-like disorder induced by reserpine administration. *Front Behav Neurosci*. 2021;15:650833.

27. Miri AL, Hosni AP, Gomes JC, Kerppers II, Pereira MCS. Estudo do L-triptofano na depressão ocorrida pela doença de Alzheimer em modelos experimentais. *J Phys Educ*. 2017;28(1):e-2839.

28. Lee H, Im J, Won H, Kim JY, Kim HK, Kwon JT, et al. Antinociceptive effect of Valeriana fauriei regulates BDNF signaling in an animal model of fibromyalgia. *Int J Mol Med*. 2018;41(1):485-92.

29. Sohroforouzani AM, Shakerian S, Ghanbarzadeh M, Alaei H. Effect of forced treadmill exercise on stimulation of BDNF expression, depression symptoms, tactile memory and working memory in LPS-treated rats. *Behav Brain Res*. 2022;418:113645.

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Should duloxetine be added to exercise to treat sedentary patients with painful knee osteoarthritis? A pilot study[a]

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ABSTRACT

Introduction: In knee osteoarthritis patients that benefit from chronic pain management and physical activity, the additional impact of duloxetine over and above exercise is yet to be determined. Our goal was to study the effects of duloxetine on muscle mass, strength, physical performance, pain, stiffness and physical function in sedentary patients with painful knee osteoarthritis treated with a home-based exercise (HE) program.

Methods: Adults with painful knee osteoarthritis and lower physical performance were assigned to receive duloxetine (60mg/d) or placebo, in addition to HE therapy. The primary endpoint was the difference in short physical performance battery (SPPB) between groups at week 12. Secondary endpoints included 12-week changes in muscle mass by dual-energy X-ray absorptiometry (appendicular skeletal muscle mass index – ASMI), strength by handgrip (HG) and knee extension (KE) maximal isometric voluntary contraction, pain by visual analog scale (VAS) and pain, stiffness and physical function by Western Ontario McMaster Universities (WOMAC) questionnaire.

Results: Twenty-four participants were included. After 12 weeks, HE+duloxetine showed no benefit in SPPB when compared to HE+placebo (p=0.456) and both groups significantly improved SPPB when compared to baseline [HE+duloxetine: 1.52 (95%CI 0.53 to 2.51); HE+placebo: 2.00 (95%CI 1.23 to 2.77)]. Both groups significantly improved WOMAC, with no differences between them (p=0.389). Only HE+duloxetine group improved pain VAS [-2.26cm (95%CI -4.08 to -0.44)], while only HE+placebo group improved ASMI [0.4Kg/m2 (95%CI 0.0 to 0.9)] and KE strength [11.8Kg (95%CI 4.3 to 19.2)]. HE+duloxetine group performed less minutes of exercise than HE+placebo group (310 vs. 692, p=0.015). Adverse events rates were similar between groups.

Conclusions: Duloxetine did not additionally improve physical performance, pain, stiffness and physical function of patients with lower physical performance and painful KOA treated with exercise. Muscle mass and muscle strength gains were only observed in the placebo group perhaps due to greater exercise adherence, but larger studies are needed to address this hypothesis.

Keywords: *Osteoarthritis; Pain; Sarcopenia*

[a] The study protocol was retrospectively registered at Registro Brasileiro de Ensaios Clínicos (ReBEC) under the identifier RBR-9c72hyz Effects of Duloxetine on muscle loss associated with knee arthrosis (https://ensaiosclinicos.gov.br/rg/RBR-9c72hyz) and universal trial number (UTN code): U1111-1259-3956. The ReBEC registration submission date was 20/02/2017 (before firstparticipant enrollment) and final approval was on 01/04/2021.

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INTRODUCTION

Osteoarthritis is the most common chronic joint disease, and pain is the most dominant symptom and the major drive of clinical decision making[1,2]. As the disease progresses, joint nociception, marked by structural changes, synovitis, nerve growth and neovascularization, gives rise to peripheral and central sensitization[1,3].

Due to its major role in chronic refractory pain, central sensitization has been considered a potential osteoarthritis treatment target[4,5]. Approved for painful conditions associated with central sensitization, duloxetine, a selective serotonin and norepinephrine reuptake inhibitor, is also recommended for chronic pain management in osteoarthritis patients[6].

Pain in osteoarthritis has also been associated with sarcopenia, a condition frequently caused or aggravated by osteoarthritis[7-9]. Defined as a progressive and generalized skeletal muscle disorder associated with greater risk of falls, fractures, physical disability and mortality[8], sarcopenia shares several pathophysiological mechanisms with osteoarthritis[9]. Mainly, osteoarthritis and sarcopenia are associated with inflammaging, a phenomenon characterized by excess secretion of proinflammatory cytokines, increase of oxidative stress, decrease in autophagy capacity and DNA damage response[10].

While in osteoarthritis patients greater pain intensity has been associated with lower muscle strength and mass[7,11-14], and duloxetine and exercise are expected to improve pain and physical function[6,15], the potential benefits of central sensitization drug therapy over and above exercise still need clarification. Therefore, our goal was to investigate the additional effects of duloxetine on physical performance, muscle mass, muscle strength, pain, stiffness and physical function of sedentary patients with painful knee osteoarthritis (KOA) treated with a home-based exercise (HE) program.

METHODS

Design, participants and settings

We conducted a double-blind parallel-group placebo controlled 12-week randomized clinical trial at a public tertiary care university teaching hospital from 2017 through 2020. The present work is being reported according to the CONSORT guidelines[16].

Participants from a terciary university hospital´s outpatient clinics and community-dwelling adults were invited to participate. Those considered eligible was randomized on a 1:1 ratio, in blocks of 4, to receive HE and duloxetine or HE and placebo. Inclusion criteria were age between 40 and 75 years old, diagnosis of KOA according to the American College of Rheumatology classification criteria[17], low/moderate physical performance defined as a short physical performance battery (SPPB) less than or equal to 9, history of knee pain for at least 3 months, moderate/high knee pain in the last week measured by a visual analog scale (VAS) greater than or equal to 40 out of 100 and sedentary lifestyle defined as less than 20 minutes of physical activity per week. Exclusion criteria were widespread pain, depressive symptoms (geriatric depression scale greater than or equal to 6), conditions other than osteoarthritis that impaired muscle function, body mass index (BMI) less than or equal to 22 Kg/m^2, use of systemic corticosteroids, nonsteroidal anti-inflammatory drugs, immunosuppressants, antidepressants, glucosamine, chondroitin, diacerein, doxycycline, avocado soybean unsaponifiables, *Harpagophytum procumbens*, capsaicin topical, new bisphosphonate in the last 6 months, any joint injection or surgery in the last 6 months, any knee prosthesis, any malignancy in the last 5 years, current or previous smoking, any cardiovascular condition except for controlled systemic arterial hypertension, liver cirrhosis or elevation of aspartate or alanine aminotransferase above three times the upper limit of normal, endogenous creatinine clearance or glomerular filtration rate less than or equal to 30ml/min, unable to adhere to study interventions or to sign written informed consent.

Interventions

Participants were randomized to receive either duloxetine 60mg orally once a day (preferably in the morning with a glass of water independent of meals, according to label instructions) or placebo, plus resistance and flexibility exercise program to be performed for at least 30 minutes three times a week at home by both groups. This HE program was a compilation of previous studies protocols aiming at improving elderly physical performance and lower limbs muscle strength[18-21]. Basically, there were 19 exercises to be performed as 10-15 repetitions (chair stand with hands support; leg raise lying on back; leg raise lying aside; knee flexion lying ventrally; feet dorsiflexion while sitting; chair stand without hands support; knee extension while sitting; knee elevation while sitting; knee flexion while standing with hands on the wall; lateral leg rise while standing with hands on the wall; trunk flexion while lying on the back; squatting; hip extension while lying on the back with flexed knees; leg and arm rise with hands and knees on the floor; rise trunk while lying ventrally with elbows and feet on the floor; staying on toes while standing; staying on the toes of one foot while standing with hands on the wall; staying on heels while standing without hands support; staying on the toes of one foot while standing without hands on the wall) and 10 exercises to be executed during 20-30 seconds (standing on side-by-side feet without support;

heel-to-toe walk with support; walk sideways with support; standing on one leg with support; heel-to-toe walk without support; walk backwards with support; walk sideways without support; standing on one leg without support; walk over short obstacles; walk backwards without support).

Study Procedures and Follow-up

After randomization (baseline), participants were seen every 4 weeks through week 12. Study appointments were confirmed by phone calls and, whenever a participant could not be reached or did not show up, another phone call was made to reschedule the visit. At each study visit, participants were evaluated for every outcome measure and received a new drug bottle and a new 30-minute set of exercises. Each set of exercises comprised a group of 5 to 10 exercises with an individualized level of complexity according to each participant's abilities. During each visit, participants were engaged in exercise simulation and an illustrated handout was given containing written recommendations for each exercise, including training description and repetitions. In the same handout, there was an exercise log, where participants had to take notes of the days they performed exercises and the time spent during each training session. Besides the registration of frequency and duration of each training session, all participants received a phone call between visits to help with eventual difficulties and assure exercise adherence. Exercise intensity should be perceived at most as "somewhat hard"[22] and it should be stopped in the presence of unbearable pain. Throughout the study, exercise difficulty was increased according to each participant's tolerability.

Outcomes and measurements

The primary study's outcome was to evaluate the effect of duloxetine on physical performance, measured by SPPB, at week 12. SPPB includes three physical performance domains: balance, walking speed and five-time sit-to-stand test[23,24]. SPPB score ranges from 0 through 12 and smaller values mean worse physical performance.

Additionally, the secondary outcomes were to investigate the effects of duloxetine on muscle mass measured by dual-energy X-ray absorptiometry (DXA), muscle strength measured by handgrip and knee extension strength tests, knee pain by VAS, knee pain, stiffness and physical function by Western Ontario McMaster Universities (WOMAC) questionnaire[8,25,26]. WOMAC score ranges from 0 through 68 and greater values mean worse osteoarthritis symptoms.

Muscle mass was evaluated by DXA (Lunar Prodigy Primo, GE Medical Systems). Appendicular skeletal muscle mass index (ASMI) was determined by the sum of arm muscles and leg muscles divided by height squared[8,25].

Handgrip strength was measured using a handheld dynamometer (Jamar Hydraulic Hand Dynamometer, Preston, USA). The participant was instructed to squeeze the handle as hard as possible for 5 seconds, 3 times with 60-second rest intervals among them, and the maximal isometric voluntary contraction (MIVC) of each hand was thus quantified. The measurement was repeated after a recovery period of 60 seconds, and the mean value of the highest score from each side was used in the analysis[25]. Knee extension strength was assessed by MIVC using a knee extension chair with a portable digital dynamometer (SKDD-100, Central Brasil Instrumentos, São Paulo, Brazil). After sitting on a knee extension chair and resting the affected limb at a knee flexion angle of 60 degrees, the participant was instructed to perform 3 times a 5-second maximal quadriceps contraction with a 3-minute resting interval among them. The mean value of the greatest score from each side was used in the analysis[27].

Ethics approval and consent to participate

The study protocol was approved in 2015 by the institutional review board of the authors' affiliated hospital. All participants were above 16 years of age and signed written informed consent before entering the study. All of the procedures were in accordance with the The Code of Ethics of the World Medical Association (Declaration of Helsinki).

Sample size and statistical analysis

Considering a clinically significant mean difference for SPPB of 0.8 (standard deviation of 0.7), 12 participants per group (n = 24) would be necessary to reject the null hypothesis with a power of 80% and an alpha of 0.05 (bicaudal)[21,28]. Sample size calculation was performed using the software WinPepi v.11.46. Considering the lack of similar studies in the literature and the relatively small sample size the present calculation yielded, a pilot study designation was deemed more suitable.

Quantitative variables were described as means and standard deviations/errors or 95% confidence intervals (95%CI) and categorical variables were described as absolute and relative frequencies. Sample distribution was assessed by Shapiro-Wilk's test. For mean comparisons, Student's t test was used, and for proportions comparisons, Pearson's chi-squared or Fisher's exact test was used. For concomitant time and group effects analysis, generalized estimating equations (GEE) were used complemented by least significant difference (LSD) test. For variables with symmetrical distributions, linear model was applied, and, in case of asymmetry, gamma model was used. All tests were 2-sided and a p value of less than 0.05 was considered significant. All statistical analyses were performed in SPSS (IBM), v. 21.0, as intention to treat.

Randomization and blinding

Each participant was randomly assigned to one of two groups: HE + duloxetine or HE + placebo. Randomization was performed in blocks of 4 participants to minimize the potential seasonal impact of the cold weather on exercise adherence. A research assistant who was not a member of the study team was responsible for randomization and drug bottles labeling. Firstly, a random sequence of twenty-four three-digit codes was generated at www.randomization.com. Each code was sealed inside an opaque envelope and the envelopes were sequentially numbered from 1 through 24 according to the original series. The three-digit codes were the participants' codes. Afterwards, another sequence of group allocation (group 1 for HE + duloxetine and group 2 for HE + placebo) was generated at the same website in six blocks of four (two codes for each group in a single block), as an example: Block 1: 1-1-2-2; Block 2: 1-2-2-1; etc. The two lists were, then, matched so that the first three-digit participant code corresponded to the first group code and so forth up to the twenty-fourth code. Finally, the blinded research assistant labeled drug bottles with the three-digit codes according to the group allocation obtained from the sequence match. Accordingly, each participant had a set of three drug bottles with 30 capsules of duloxetine 60 mg or placebo per bottle. Randomization and labeling were performed at the beginning of the study and drug bottles were stored in a specific locked research cabinet. When a new participant was included, a member of the study team opened the next envelope, picked up the respective labelled drug bottle from the research cabinet and handed it over to the participant. All participants and members of the research team were blinded to group allocation throughout the study.

RESULTS

Between March 2017 and January 2020, 24 subjects were randomly assigned to receive either HE+duloxetine or HE+placebo, and 14 complete the study (overall dropout rate: 41.7%) (Figure 1). Participants mean age was 64.4 years old and predominantly white (79.2%), women (77.2%) with moderate/severe radiographic KOA (86.4%) (Table 1). There were 4 participants (2 per group) that withdrew the study due to adverse events (18.2%) and 6 participants were lost to follow-up (2 from HE + duloxetine group and 4 from HE + placebo group: 16.7% vs. 33.3%, respectively; p = 0.640).

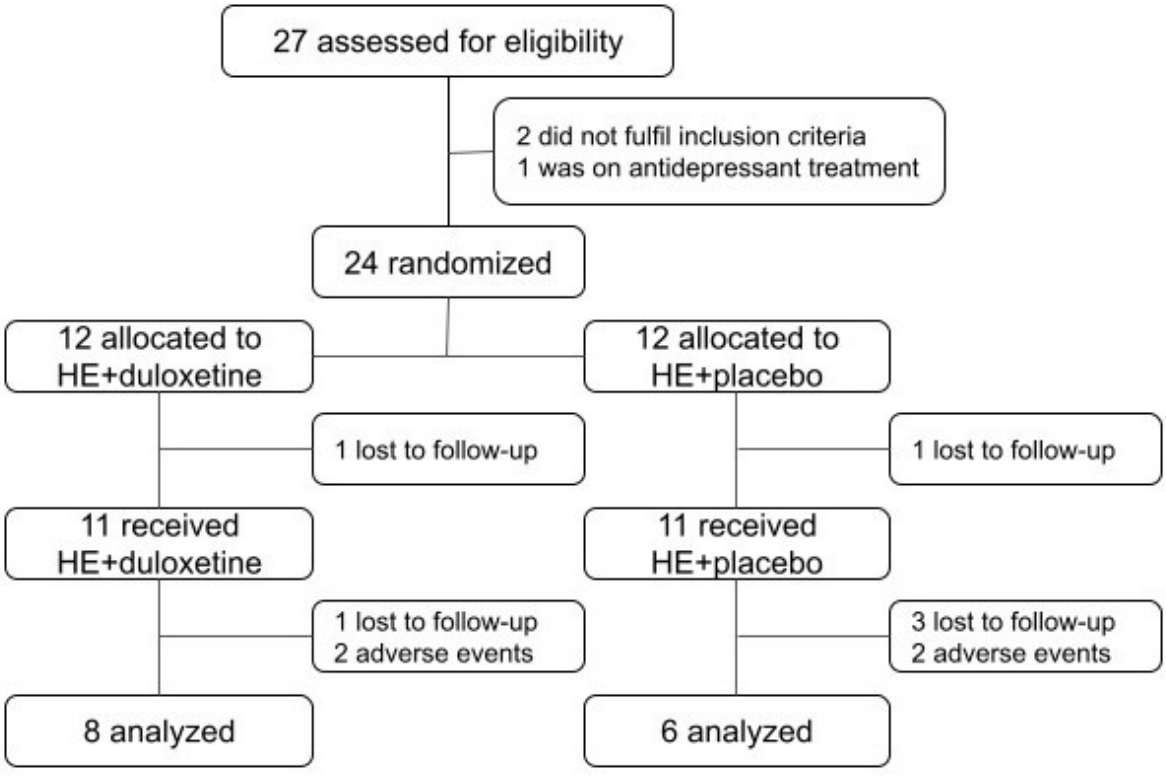

Figure 1: Participants' flowchart.
HE: home-based exercise

Table 1: Baseline participants' characteristics.

	HE+duloxetine (n=11)	HE+placebo (n=11)	p
Age (years)	64.8 ± 6.4	63.9 ± 7.3	0.760
Skin color			0.035
White	11 (100)	6 (54.5)	
Sex			1.000
Women	9 (81.8)	8 (72.7)	
Affected side of KOA			0.305
Right	5 (45.5)	2 (18.2)	
Left	1 (9.0)	3 (27.3)	
Bilateral	5 (45.5)	6 (54.5)	
Kellgren & Lawrence scale			0.420
I	0 (0.0)	0 (0.0)	
II	2 (18.2)	1 (9.1)	
III	3 (27.3)	6 (54.5)	
IV	6 (54.5)	4 (36.4)	
BMI (kg/m^2)	31.4 ± 6.8	29.9 ± 4.4	0.548
Vitamin D, serum (ng/mL)	22.5 ± 10.1	24.7 ± 7.7	0.574
Pain VAS (0 to 10)	7.1 ± 1.2	6.3 ± 2.2	0.328
WOMAC total	50.3 ± 19.2	53.8 ± 9.6	0.593

Values as mean ± SD or n(%).

HE: home-based exercise; KOA: knee osteoarthritis; BMI: body mass index; VAS: visual analog scale; WOMAC: Western Ontario McMaster Universities questionnaire

After 12 weeks, HE + duloxetine group showed no benefit in SPPB when compared to HE + placebo group (p = 0.456) and both groups significantly improved SPPB when compared to baseline [HE + duloxetine: 1.52 (95%CI 0.53 to 2.51); HE + placebo: 2.00 (95%CI 1.23 to 2.77)] (Table 2).

Table 2: Comparisons of SPPB score along the study according to group.

SPPB	HE+ Duloxetine		HE+Placebo		p value
	n	Mean ± SE	n	Mean ± SE	
Balance component (0-4)					
Baseline	11	3.73 ± 0.13^{a}	11	3.45 ± 0.15^{a}	0.176
W4	10	3.70 ± 0.20^{ab}	10	4.00 ± 0.00^{b}	0.138
W8	9	3.67 ± 0.16^{a}	7	3.71 ± 0.17^{ab}	0.837
W12	8	4.00 ± 0.00^{b}	6	3.83 ± 0.15^{ab}	0.273
Variation (12w-Baseline)		0.27 (0.01 to 0.54)		0.38 (-0.10 to 0.86)	0.706
p value		0.042		0.125	
Walking speed component (0-4)					
Baseline	11	2.91 ± 0.35^{a}	11	3.27 ± 0.26^{a}	0.405
W4	10	3.10 ± 0.33^{a}	10	3.50 ± 0.21^{a}	0.308
W8	9	3.11 ± 0.25^{a}	7	3.71 ± 0.17^{a}	0.044
W12	8	3.13 ± 0.28^{a}	6	3.67 ± 0.19^{a}	0.107
Variation (W12-Baseline)		0.22 (-0.49 to 0.92)		0.39 (-0.14 to 0.93)	0.693
p value		0.547		0.149	
FTSTST component (0-4)					
Baseline	11	1.09 ± 0.16^{a}	11	1.27 ± 0.19^{a}	0.453
W4	10	1.90 ± 0.26^{b}	10	1.60 ± 0.21^{b}	0.372
W8	9	2.00 ± 0.27^{b}	7	2.00 ± 0.35^{bc}	1.000
W12	8	2.13 ± 0.28^{b}	6	2.50 ± 0.31^{c}	0.368
Variation (W12-Baseline)		1.03 (0.33 to 1.74)		1.23 (0.44 to 2.02)	0.720
p value		0.004		0.002	
Total (0-12)					
Baseline (n=11)	11	7.73 ± 0.45^{a}	11	8.00 ± 0.22^{a}	0.585
W4 (n=10)	10	8.70 ± 0.68^{bc}	10	9.10 ± 0.30^{b}	0.590

Continues...

Table 2: Continuation.

SPPB	HE+ Duloxetine		HE+Placebo		p value
	n	Mean ± SE	n	Mean ± SE	
W8 (n=9)	9	8.78 ± 0.52[b]	7	9.43 ± 0.45[b]	0.340
W12 (n=8)	8	9.25 ± 0.42[c]	6	10.0 ± 0.41[c]	0.203
Variation W12-Baseline (CI 95%)		1.52 (0.53 to 2.51)		2.00 (1.23 to 2.77)	0.456
p value		0.003		<0.001	

[a,b,c,d] Same letters do not differ according to Least Significant Difference (LSD) test at 5% of significance level

SPPB: short physical performance battery; HE: home-based exercise; SE: standard error; FTSTST: five-time sit-to-stand test

HE + duloxetine group performed less minutes of exercise than HE+placebo group [310 (IQR 333) vs. 692 (IQR 820), p = 0.015]. Both groups significantly improved WOMAC, with no differences between them (p = 0.389) (Table 3).

Only HE + duloxetine group improved pain VAS [- 2.26 cm (95%CI - 4.08 to - 0.44)] and the balance component of SPPB [0.27 (95%CI 0.01 to 0.54)], while only HE+placebo group improved ASMI [0.4 Kg/m2 (95%CI 0.0 to 0.9)] and knee extension strength [11.8 Kg (95%CI 4.3 to 19.2)] (Tables 2, 3 and 4). Handgrip strength did not change significantly throughout the study in either group (Table 4). Overall, intra-group significant differences were not complemented by inter-group statistical significance.

In total, 10 (45.5%) patients experienced any adverse event throughout the study: 6 (27.3%) mild/moderate and 4 (18.2%) severe adverse events (2 participants in each group). Numerically, more patients in HE + duloxetine group than in the HE + placebo group had mild/moderate adverse events without statistical significance [4 (36.4%) vs. 2 (18.2%), *p*=0.598].

Table 3: Comparisons of pain visual analog scale and WOMAC score along the study according to group.

	HE+Duloxetine		HE+Placebo		p value
	n	Mean ± SE	n	Mean ± SE	
Pain VAS (0-10cm)					
Baseline	11	7.08 ± 0.35[b]	11	6.32 ± 0.64[b]	0.293
W4	10	3.84 ± 0.88[a]	9	4.78 ± 0.88[a]	0.012
W8	9	4.42 ± 0.70[a]	6	4.72 ± 1.33[ab]	0.844
W12	8	4.68 ± 0.78[a]	6	4.35 ± 0.91[ab]	0.786
Variation W12-Baseline (CI 95%)		-2.41 (-3.82 to -0.99)		-1.97 (-4.40 to 0.47)	0.760
p value		0.001		0.113	
WOMAC – Pain (0-20)					
Baseline	11	10.6 ± 1.19[c]	11	10.6 ± 0.95[c]	1.000
W4	11	6.18 ± 0.96[a]	10	9.40 ± 1.32[b]	0.049
W8	9	8.56 ± 1.31[b]	6	7.67 ± 1.94[ab]	0.704
W12	8	8.38 ± 1.33[b]	6	6.50 ± 1.35[a]	0.323
Variation W12-Baseline (CI 95%)		-2.26 (-4.08 to -0.44)		-4.14 (-5.90 to -2.38)	0.147
p value		0.015		<0.001	
WOMAC – Stiffness (0-8)					
Baseline	11	3.82 ± 0.49[a]	11	4.36 ± 0.67[a]	0.513
W4	11	3.27 ± 0.48[a]	10	2.90 ± 0.54[a]	0.606
W8	9	3.67 ± 0.72[a]	6	3.50 ± 0.81[a]	0.878
W12	8	3.38 ± 0.93[a]	6	3.50 ± 0.94	0.925
Variation W12-Baseline (CI 95%)		-0.44 (-1.50 to 0.61)		-0.86 (-2.49 to 0.76)	0.671
p value		0.412		0.298	
WOMAC – Physical function (0-68)					
Baseline	11	35.8 ± 4.36[b]	11	38.8 ± 2.25[b]	0.541
W4	11	24.0 ± 4.72[a]	10	34.3 ± 3.65[b]	0.084
W8	9	25.1 ± 5.47[a]	6	20.8 ± 5.91[a]	0.595
W12	8	25.9 ± 6.08[a]	6	25.3 ± 4.64[a]	0.944
Variation W12-Baseline (CI 95%)		-9.94 (-17.5 to -2.44)		-13.5 (-22.0 to -4.99)	0.540
p value		0.009		0.002	

Continues...

Table 3: Continuation.

	HE+Duloxetine		HE+Placebo		p value
	n	Mean ± SE	n	Mean ± SE	
WOMAC – Total (0-96)					
Baseline	11	50.3 ± 5.52	11	53.8 ± 2.77	0.566
W4	11	33.5 ± 5.56	10	46.6 ± 4.93	0.077
W8	9	37.3 ± 7.12	6	32.0 ± 8.17	0.622
W12	8	37.6 ± 7.88	6	35.3 ± 6.57	0.823
Variation W12-Baseline (CI 95%)		-12.7 (-21.3 to -3.99)		-18.5 (-28.6 to -8.41)	0.389
p value		0.004		<0.001	

[a,b,c,d] Same letters do not differ according to *Least Significant Difference* (LSD) test at 5% of significance level

WOMAC: Western Ontario McMaster Universities; VAS: visual analog scale; HE: home-based exercise; SE: standard error

Table 4: Comparisons of appendicular skeletal muscle mass index and handgrip and knee extension strength along the study according to group.

	HE+Duloxetine		HE+Placebo		p value
	n	Mean ± SE	n	Mean ± SE	
Appendicular skeletal muscle mass index (Kg/m^2)					
Baseline	11	7.1 ± 0.3	11	7.8 ± 0.4	0.170
W12	8	7.2 ± 0.3	6	8.2 ± 0.4	0.021
Variation (W12-Baseline)		0.1 (-0.5 to 0.5)		0.4 (0.0 to 0.9)	0.204
p value		0.924		0.044	
Handgrip strength (Kg)					
Baseline	11	21.8 ± 1.9[a]	11	27.1 ± 3.8[a]	0.209
W4	11	23.8 ± 2.0[a]	10	27.8 ± 4.4[a]	0.404
W8	10	23.8 ± 2.0[a]	8	32.1 ± 4.9[a]	0.114
W12	8	22.6 ± 2.0[a]	6	32.7 ± 5.0[a]	0.061
Variation W12-Baseline (CI 95%)		0.8 (-3.6 to 5.3)		5.6 (-1.3 to 12.6)	0.482
p value		0.723		0.113	
Knee extension strength (Kg)					
Baseline	11	15.7 ± 2.8[a]	11	19.5 ± 3.4[a]	0.384
W4	11	15.0 ± 2.7[a]	10	23.1 ± 3.6[ab]	0.070
W8	10	16.8 ± 3.1[a]	8	27.6 ± 5.1[bc]	0.068
W12	8	16.9 ± 3.2[a]	6	31.3 ± 4.8[c]	0.013
Variation W12-Baseline (CI 95%)		1.2 (-2.3 to 4.6)		11.8 (4.3 to 19.2)	0.063
p value		0.506		0.002	

[a,b,c,d] Same letters do not differ according to *Least Significant Difference* (LSD) test at 5% of significance level

HE: home-based exercise; SE: standard error

DISCUSSION

To the best of our knowledge, this is the first study designed to assess the effects of chronic pain management on sarcopenia in patients with KOA. According to our findings, duloxetine was not superior to placebo in improving physical performance of sedentary patients with painful KOA treated with exercise. Both groups equally improved SPPB and WOMAC throughout the 12-week period of follow-up. There was a significant improvement in pain VAS only in HE + duloxetine group, but that was not accompanied by muscle mass or muscle strength increment. Interestingly, muscle mass and muscle strength were significantly better only in HE + placebo group.

Despite the reported associations between pain and muscle mass or strength in KOA, the overall association between pain and sarcopenia remains elusive. Allegedly, knee pain could be associated with either muscle mass, muscle strength or physical performance at most in subgroups of KOA patients. In the study by Scott, et al., greater knee pain predicted lower leg strength and muscle quality only in women[7]. In the KNHANES study, lower muscle mass was independently associated with greater knee pain only in radiographically mild KOA patients[14]. In the SPSS-OK study, pain was not associated with sarcopenia, defined by muscle mass, handgrip strength and walking speed[29], but could be moderating the association between muscle mass and muscle strength in radiographically severe KOA patients[30].

Our results indicate that exercise improves function in KOA patients and this finding is consistent with the

literature[6]. However, the effect of exercise in muscle mass and muscle strength could only be appreciated without duloxetine. Also, there was a greater amount of time dedicated to home exercise in the placebo group. This difference could mainly have been due to three reasons. First, pain improvement induced by duloxetine could have been interpreted as an exemption from strict exercise adherence, since, during study visits, both interventions were mentioned as associated with symptoms amelioration. Second, although not statistically different, a numerically greater number of milder adverse events in duloxetine users, such as nausea and dizziness, could have played a role in refraining from exercising. In other words, duloxetine-induced adverse events could have impaired exercise adherence, but our sample was probably not large enough to demonstrate a true difference of adverse events between groups. Despite the small sample size, results were according to a parametric distribution, because Shapiro-Wilk's test was not statistically significant and there was homoscedasticity in both groups. Finally, the greater number of participants lost to follow-up in the placebo group could have been due to lack of efficacy and the observed differences in muscle mass and strength subjected to attrition bias.

The present study has some limitations. Firstly, despite achieving the estimated sample size, our results could have been affected by a greater than expected dropout rate, which raises the possibility of a type 2 error[31]. Also, the substantial number of losses to follow-up prevented any inference of the direction of the treatment effect and any conclusion needs studies with larger sample sizes. Notwithstanding our attempt to sustain study endurance by regular phone calls, the high number of participants lost to follow-up could be attributed to the greater mobility impairment inherent to a clinical scenario that combines low physical performance with painful KOA at baseline. Futures studies should consider a greater than expect dropout rate to circumvent this relevant limitation. To minimize the biases of losses to follow-up, we used GEE, that allows the inclusion of every participant in the analysis, not only those that completed the study[32]. In addition, although the study was designed to find a clinically significant difference in SPPB, our final sample size was not large enough to allow any subgroup or regression analysis. Therefore, the longitudinal association between pain and sarcopenia, as well as how this interaction could be moderated by sex, body composition and radiographic osteoarthritis severity could not be properly addressed herein. At last, the numerous exclusion criteria adopted to assure participants' safety before study interventions ultimately limited the generalizability of our findings to different populations.

In the present study, duloxetine did not additionally improve physical performance, pain, stiffness and physical function of patients with lower physical performance and painful knee osteoarthritis treated with exercise. Regarding pain, only duloxetine group showed a statistically significant improvement during the 12-week follow-up, even though this variation was not statistically different from the variation observed in the placebo group. Muscle mass and muscle strength gains were only observed in the placebo group perhaps due to exercise adherence issues in the duloxetine group and/or attrition bias, even though we were not able to demonstrate any of these inferences, due to a limited sample size. For these reasons, our results are not enough to change clinical practice protocols towards duloxetine prescription intended to improve physical function, muscle mass or pain management in KOA patients. Therefore, larger studies are needed to specify the eventual additional benefits of central sensitization drug therapy over and above exercise in an osteoarthritis population that benefits from both interventions.

Declarations

Ethics approval and consent to participate

The study protocol was approved in 2015 by local ethics committee (Comitê de Ética em Pesquisa do Grupo de Pesquisa e Pós-Graduação do Hospital de Clínicas de Porto Alegre – 15-0549; CAAE: 50785715000005327). All participants were above 16 years of age and signed written informed consent before entering the study. All of the procedures were in accordance with the Helsinki Declaration.

Availability of data and materials

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

Competing interests

The authors declare that they have no conflicts of interest/competing interests.

Conflicts of interest: none.

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Authors' Contributions

RMDSC participated in the conception of the work, acquisition, analysis and interpretation of data, drafting and critically revising the work. RCDES, KPS and MMDS participated in the acquisition, analysis and interpretation of data, and critically revising the work. LPDS participated in the acquisition and interpretation of data, and critically revising the work.

MEDM, JB, PSC, AAG and VH participated in the acquisition and interpretation of data, and critically revising the work. All authors read and approved the final manuscript.

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REFERENCES

1. Fu K, Robbins SR, McDougall JJ. Osteoarthritis: the genesis of pain. *Rheumatology (Oxford)*. 2018;57(suppl_4):iv43-50.
2. Neogi T. The epidemiology and impact of pain in osteoarthritis. *Osteoarthritis Cartilage*. 2013;(9):1145-53.
3. Hawker GA, Stewart L, French MR, Cibere J, Jordan JM, March L, et al. Understanding the pain experience in hip and knee osteoarthritis--an OARSI/OMERACT initiative. *Osteoarthritis Cartilage*. 2008;(4):415-22.
4. Havelin J, Imbert I, Cormier J, Allen J, Porreca F, King T. Central sensitization and neuropathic features of ongoing pain in a rat model of advanced osteoarthritis. *J Pain*. 2016;17(3):374-82.
5. Pettersen PS, Neogi T, Magnusson K, Hammer HB, Uhlig T, Kvien TK, et al. Peripheral and central sensitization of pain in individuals with hand osteoarthritis and associations with self-reported pain severity. *Arthritis Rheumatol*. 2019;71(7):1070-7.
6. Kolasinski SL, Neogi T, Hochberg MC, Oatis C, Guyatt G, Block J, et al. 2019 American College of Rheumatology/ Arthritis foundation guideline for the management of osteoarthritis of the hand, hip, and knee. *Arthritis Care Res (Hoboken)*. 2020;72(2):149-62.
7. Scott D, Blizzard L, Fell J, Jones G. Prospective study of self-reported pain, radiographic osteoarthritis, sarcopenia progression, and falls risk in community-dwelling older adults. *Arthritis Care Res*. 2012;64(1):30-7.
8. Cruz-Jentoft AJ, Bahat G, Bauer J, Boirie Y, Bruyère O, Cederholm T, et al. Sarcopenia: Revised European consensus on definition and diagnosis. *Age Ageing*. 2019;48(1):16-31.
9. De Ceuninck F, Fradin A, Pastoureau P. Bearing arms against osteoarthritis and sarcopenia: When cartilage and skeletal muscle find common interest in talking together. *Drug Discov Today*. 2014;19(3):305-11.
10. Rezus E, Cardoneanu A, Burlui A, Luca A, Codreanu C, Tamba BI, et al. The link between inflammaging and degenerative joint diseases. *Int J Mol Sci*.2019;20(3):614.
11. Wang Y, Wluka AE, Berry PA, Siew T, Teichtahl AJ, Urquhart DM, et al. Increase in vastus medialis cross-sectional area is associated with reduced pain, cartilage loss, and joint replacement risk in knee osteoarthritis. *Arthritis Rheum*. 2012;64(12):3917-25.
12. Cheon YH, Kim HO, Suh YS, Kim MG, Yoo WH, Kim RB, et al. Relationship between decreased lower extremity muscle mass and knee pain severity in both the general population and patients with knee osteoarthritis: findings from the KNHANES. *PLoS One*. 2017;12(3):e0173036.
13. Lee JY, Han K, McAlindon TE, Park YG, Park SH. Lower leg muscle mass relates to knee pain in patients with knee osteoarthritis. *Int J Rheum Dis*. 2018;21(1):126-33.
14. Park HM, Kim HJ, Lee B, Kwon M, Jung SM, Lee SW, et al. Decreased muscle mass is independently associated with knee pain in female patients with radiographically mild osteoarthritis: a nationwide cross-sectional study (KNHANES 2010-2011). *Clin Rheumatol*. 2018;37(5):1333-40.
15. Gao SH, Huo JB, Pan QM, Li XW, Chen HY, Huang JH. The short-term effect and safety of duloxetine in osteoarthritis: a systematic review and meta-analysis. *Medicine (Baltimore)*. 2019;98(44):e17541.
16. Schulz KF, Altman DG, Moher D, CONSORT Group. CONSORT 2010 Statement: updated guidelines for reporting parallel group randomised trials. BMJ. 2010;340:c332.
17. Altman R, Asch E, Bloch D, Bole G, Borenstein D, Brandt K, et al. Development of criteria for the classification and reporting of osteoarthritis. Classification of osteoarthritis of the knee. Diagnostic and Therapeutic Criteria Committee of the American Rheumatism *Association. Arthritis Rheum*. 1986;29(8):1039-49.
18. Abou-Raya S, Abou-Raya A, Kareem AA. SAT0314 Effects of Physical Activity on Inflammation, Skeletal Muscle Strength/Function (Sarcopenia) and Fat Infiltration (Sarcopenic Obesity) in Older Adults With Knee Osteoarthritis: A Randomized Controlled Trial. *Ann Rheum Dis*. 2013;72:A690.
19. Clegg A, Barber S, Young J, Iliffe S, Forster A. The Home-based Older People's Exercise (HOPE) trial: a pilot randomised controlled trial of a home-based exercise intervention for older people with frailty. *Age Ageing*. 2014;43(5):687-95.
20. Brown M, Sinacore DR, Ehsani AA, Binder EF, Holloszy JO, Kohrt WM. Low-Intensity exercise as a modifier of physical frailty in older adults. *Arch Phys Med Rehabil*. 2000;81(7):960-5.
21. Marsh AP, Chmelo EA, Katula JA, Mihalko SL, Rejeski WJ. Should physical activity programs be tailored when older adults have compromised function? *J Aging Phys Act*. 2009;17(3):294-306.
22. Borg GA. Psychophysical bases of perceived exertion. *Med Sci Sport Exerc*. 1982;14(5):377-81.
23. Guralnik JM, Ferrucci L, Simonsick EM, Salive ME, Wallace RB. Lower-extremity function in persons over the age of 70 years as a predictor of subsequent disability. *N Engl J Med*. 1995;332(9):556-61.
24. Nakano MM. *Versão brasileira da Short Physical Performance Battery SPPB: adaptação cultural e estudo da confiabilidade* [master's thesis]. Campinas: Faculdade de Educação, Universidade Estadual de Campinas; 2007. 181 p.
25. Santo RC, Silva JM, Lora PS, Moro ALD, Freitas EC, Bartikoski BJ, et al. Cachexia in patients with rheumatoid arthritis: a cohort study. *Clin Rheumatol*. 2020;39(12):3603-13.

26. Fernandes MI. *Translation and validation of the specific quality of life questionnaire for osteoarthritis WOMAC (Western Ontario McMaster Universities) for portuguese language* [master's thesis]. São Paulo: Escola Paulista de Medicina, Universidade Federal de São Paulo; 2003. 119 p.

27. Brown LE, Weir JP, Brown LK, Weir D. ASEP procedures recommendation I: accurate assessment of muscular strength and power. *Journal of Exercise Physiology*. 2001;4(3):1-21.

28. Kwon S, Perera S, Pahor M, Katula JA, King AC, Groessl EJ, et al. What is a meaningful change in physical performance? Findings from a clinical trial in older adults (The LIFE-P study). *J Nutr Heal Aging*. 2009;13(6):538-44.

29. Kurita N, Kamitani T, Wada O, Shintani A, Mizuno K. Biomarkers and Sarcopenia in the Presence of Pain and Inflammation Among Patients With Osteoarthritis. 2019;1-8.

30. Wada O, Kurita N, Kamitani T, Nakano N, Mizuno K. Influence of the severity of knee osteoarthritis on the association between leg muscle mass and quadriceps strength: the SPSS-OK study. Clin Rheumatol. 2019;38(3):719-25.

31. Dettori J. Loss to follow-up. Evid Based Spine Care J 2011;2(1):7-10.

32. Guimaraes LSP, Hirakata VN. Use of the Generalized Estimating Equation Model in longitudinal data analysis. Rev HCPA. 2012;32(4):503-11.

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Lung ultrasonography results statistically agree with computed tomography in COVID-19 pneumonia cases from a private hospital in southern Brazil

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ABSTRACT

Introduction: Computed tomography (CT) scans can both exclude other pathological conditions and display a high level of sensitivity for COVID-19 pneumonia. Although LUS has the potential to replace these methods in patient evaluation, issues remain as to whether it can be considered a reliable surrogate in the clinical decision process.

Objective: To compare the performance of lung ultrasonography (LUS) with that of lung computed tomography (CT) scans in patients with suspected COVID-19 for the presence of interstitial pneumonia and the degree of lung injury.

Methods: In a cross-sectional clinical study, LUS and CT were compared for the presence of interstitial pneumonia and the degree of lung injury in patients with suspected COVID-19. Pearson's correlation analysis was performed to measure the degree of association between the two methods. Bland–Altman plot was applied. *P*-values ≤ 0.05 were considered significant.

Results: A good correlation between LUS and CT scan was obtained for estimates of lung injury in pneumonia in a group of COVID-19 patients ($R^2 = 0.7186$; $p < 0.01$). Agreement between LUS and CT values is assessed by constructing the Bland-Altman plot and all data points fall within ± 1.96 times the standard deviation of the difference between the results of the two methods. This corroborates that there is a strong agreement between the two methods.

Conclusions: LUS, as compared to CT scans, is an effective method to estimate degrees of lung injury in patients with suspected Covid-19 in the emergency department.

Keywords: *COVID-19; Computed Tomography; Lung Ultrasonography; Pneumonia*

INTRODUCTION

Critical care ultrasonography has gained importance in the ICU (intensive care unit) and emergency department (ED) scene over the last decade[1]. It was initially used in the evaluation of the trauma patient to disclose intra-abdominal bleeding, an approach known as the FAST examination[2]. Ultrasound-assisted deep vein puncture and bedside echocardiography follow suit. As a novel use of ultrasonography beyond the boundaries of the radiological department, the exploration of the lung somewhat lagged. The virtual absence of lung ultrasonography (LUS) uses in the realm of conventional radiology and the idea that air hampers the transmission of sound set this modality aside from the other areas of point-of-care ultrasonography. Only recently has it come of age and revealed itself as an area deserving attention in the investigation of the critically ill[3,4].

COVID-19 patients are progressively being evaluated for pulmonary compromise in emergency departments and intensive care units by LUS rather than chest XR (X-Ray) or CT (computed tomography) scan. While the

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former underperforms lung ultrasonography, the latter imposes the hindrances of patient dislocation, ionizing radiation, and waiting times for results. Although LUS has the potential to replace these methods in patient evaluation, issues remain as to whether it can be considered a reliable surrogate in the clinical decision process[5].

Decisions concerning COVID-19 patients are, firstly, whether to investigate patients presenting with respiratory symptoms in the ED and, secondly, whether discharge them from the ED or admit them to the hospital. Rarely if ever, in the course of COVID-19 disease, decisions about oxygen therapy, advanced support of respiratory function, use of anti-inflammatory drugs, anticoagulants, or antibiotics are made based on lung imaging, apart from pulmonary angiography in embolism-suspected patients. Instead, respiratory function, inflammatory markers, coagulation tests, and white blood cell counts help the clinician in therapeutic decisions. Having said that, and acknowledging that imaging is but a small part in conveying the information needed for the decision of discharging patients without investigation from the ED and about the need for admission, LUS may perform well as compared to chest XR and lung CT scans, provided that its capacity to estimate degrees of lung involvement correlate and agree with CT scans. Furthermore, US (ultrasonography) may add valuable information on alternative diagnoses of respiratory failure[6] LUS can provide quite sensitive data for lung interstitial syndrome making it a valuable alternative to other methods[7,8].

LUS could also be used in the follow-up of patients already admitted for COVID-19 pneumonia, especially when worsening of the clinical picture and lowering lung function demands an explanation. Despite its low sensitivity for interstitial syndrome, chest XR has been used on COVID-19 patients to exclude alternative diagnoses. CT scans can both exclude other pathological conditions and display a high level of sensitivity for COVID-19 pneumonia. We, therefore, decided to compare the performance of LUS with that of lung CT scans in patients with suspected COVID-19 for the presence of interstitial pneumonia and the degree of lung injury.

METHODS

In a cross-sectional clinical study, LUS and CT were compared for the presence of interstitial pneumonia and the degree of lung injury in patients with suspected COVID-19. We ranked the degree of lung compromise with the US by assigning values for pleural lesions, the intensity of B lines, and consolidation. The investigators determined the scores for both methods blinded.

For the pleural line, we attributed a value of 0 for normal, 1 for irregular pattern, and 2 for the broken line. For the presence of B lines, we attributed 0 for absent, 1 for non-confluent B lines, 2 for confluent B lines, and 3 for white lung pattern. For the presence of consolidation, we attributed 0 for absent and 1 for presence. The values obtained for each of these variables were computed for each point of the LUS examination, namely points A, B, PLAPS for the right and left lung totalizing a maximum of 36 possible points per patient. We compared these zones with topographic equivalents on CT Scans. Accordingly, CT was evaluated for ground glass opacities (GGO), consolidation and/or atelectasis, crazy paving pattern, and pleural effusion. Topographic equivalents with LUS were chosen representing in a gross mode the A, B, PLAPS points, respectively corresponding to the upper lobes, the middle lobe on the right or lingula on the left, and the lower lobes. For parenchymal opacities, in a visual scale score, we attributed value 0 for normal, 1 for up to 25% of lung compromise, 2 for 26-50%, 3 for 51-75%, and 4 for more than 75% lung compromise, applied to 6 parts of lung representing in a gross mode the A, B, PLAPS bilaterally, and to each one of the lesions. The sum was the final score, totalizing a maximum of 24 possible points per patient. We also used a specific software (Siemens Healthcare CT Pneumonia Analysis) to compare the quantitative percentage of lung compromise. Pleural effusion was considered present or absent.

The enrolment period ran from March to November 2020. Patients of both sexes, aged 18 years old and above on the day of evaluation at the ED were included. Those with previous heart and lung disease were not excluded. Active flu symptoms, fever, or desaturation not immediately explained by previous conditions were the clinical criteria for inclusion. Some of these patients were known to be positive for SARS-CoV-2 infection by previous testing. Those first tested at the department had the results available only on the days following evaluation. All participants signed an informed consent form and this study was approved by the Institutional Review Board of the National Health Council of Brazil (approval number 4.497.118).

Data were analyzed using the Statistical Package for Social Sciences (SPSS, version 23.0, Chicago, IL) and R studio available in R platform (http://www.R-project.org/). The Kolmogorov-Smirnov test was used to assess the type of distribution of quantitative variables. Pearson's correlation analysis was performed to measure the degree of association between the two methods. Bland–Altman plot was generated to provide a graphical visualization of the agreement between the two measurement methods[9]. The mean values (X-axis) were plotted against the difference between the two methods (Y-axis) to identify any systematic bias and possible outliers.

Horizontal lines were drawn at the mean difference and at the limits of agreement, defined as the mean difference plus and minus 1.96 times the standard deviation of the differences. All statistical tests in this study were two-sided and *p*-values ≤ 0.05 were considered statistically significant.

RESULTS

During the application of the protocol, 22 patients were analyzed, the data are detailed below. Male patients were 68.2% and the median age was 59 years (interquartile range [IQR]: 41.5 – 68.0). Comorbidities detected were, in order of frequency: hypertension (52.4%), unspecified cancer (23.8%), respiratory disease (19%), obesity (19%), smoking (9.5%), unspecified diabetes (4.8%), hypothyroidism (4.8%), liver disease (4.8%), immunodeficiency (4.8%), and depression/anxiety (4.8%). All patients had a positive RT-PCR test for COVID-19. During hospitalization, the median oxygen saturation of the patients was 95% (IQR: 94-98%), with a median respiratory rate of 20 respiratory movements per minute (IQR: 19-21) with 4 of these patients requiring mechanical ventilation during the period.

A good correlation by Pearson coefficient analysis between LUS and CT scan was obtained for estimates of lung injury in pneumonia in a group of COVID-19 patients ($R^2 = 0.7186$; $p < 0.01$) (**Figure 1**).

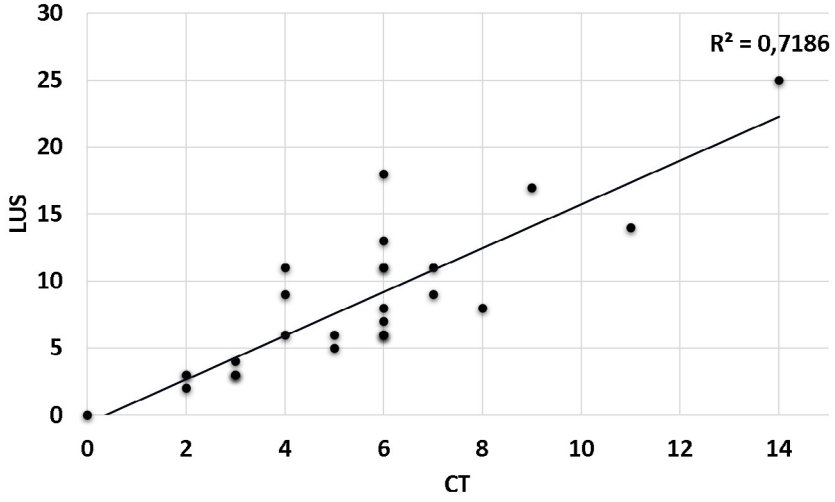

Figure 1: Pearson correlation coefficient analysis between LUS (lung ultrasonography) and CT (computed tomography).

Agreement between LUS and CT values is assessed by constructing the Bland-Altman plot (**Figure 2**). X-axis suggests the mean of LUS and CT. Y-axis was plotted by the difference between LUS and CT. All data points fall within ± 1.96 times the standard deviation of the difference between the LUS and CT values. This corroborates that there is a strong agreement between the two methods.

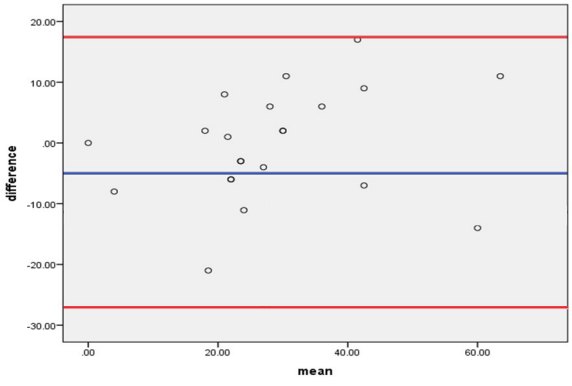

Figure 2: Bland-Altman plot for LUS (lung ultrasonography) and CT (computed tomography) results.

DISCUSSION

We studied patients being investigated at the ED for the possibility of COVID-19 infection and for clinical evaluation that resulted in discharge or admission. It is therefore unsurprising that 100 % of the patients tested positive for SARS-CoV-2 at the time of image acquisition. Some of these patients were already known to be RT-PCR positive for having been tested days before their visit to the ED. A good correlation between LUS and CT scan was obtained for estimates of lung injury in pneumonia in a group of patients with suspected COVID-19. Also, agreement between LUS and CT values was detected by Bland-Altman plot. The interstitial syndrome has been well characterized in LUS, expresses itself by the pattern of B lines, and correlates with above-normal levels of water in the lungs. Allinovi et al[10], reviewing the literature concluded that LUS can detect the interstitial lung syndrome through the evaluation and quantitation of B lines, pleural irregularities, and consolidations This syndrome is associated with intra-alveolar and interstitial fluid, a result of either hydrostatic or inflammatory edema. That said, the presence of B lines is an artifact of unspecific etiology in LUS. However, patterns of distribution amongst lung zones, like the A/B combination that suggests pneumonia, the clinical setting in which B lines are observed, namely patients with suspect heart failure, infection, or lymphangitic carcinomatosis, or, as in our series, the epidemiological context of the COVID-19 pandemic, all convey the necessary specificity for this finding to attain diagnostic utility. Accordingly, specificity increases with CO-RADS level[11].

In addition to the epidemiological context, specificity can also be affected by the definition of what is a US positive finding. Narinx et al.[12], considered US examination positive for COVID-19 when B lines were found in only one of the BLUE protocol areas. The use of a score might result in improvements in specificity. LUS has been acknowledged as high in COVID-19, whereas sensitivity scores are low[11]. However, in this meta-analysis, positivity for COVID-19 in LUS examinations is either not mentioned or constructed on limited criteria. According to the present study, specificity found by Fonsi et al[13], was significant (79%). In this context, the interstitial lung syndrome on the LUS examination of patients with a very low likelihood of alternative etiologies strongly suggests SARS-CoV-2 infection, as was the case in our series. However, not all RT-PCR-positive patients will display an abnormal LUS examination, as some patients are evaluated in the pre-pneumonic phase of their disease, and some never really develop pneumonia as part of the clinical picture of COVID-19. This explains some of the false negative results found with LUS. Accordingly, previous studies[14,15] in retrospective research with patients evaluated with LUS at the ED, found that amongst 31 RT-PCR-positive patients, 28 were detected by LUS and of 15 diagnosed by LUS, 11 were confirmed by RT-PCR positive for SARS-CoV-2. COVID-19 suggestive findings at LUS in RT-PCR negative patients may result from RT-PCR sampling in upper airways when the infection is no longer active at this site, but in the lung parenchyma, where bronchoalveolar lavage sampling may be positive[16]. Trauer et al[17] stress the importance of the background on which LUS is used for precise accuracy of LUS in COVID-19, as both sensitivity and specificity may be influenced by disease severity, pre-existing lung disease, scanning protocol, operator experience, disease prevalence, and reference standard. These limitations are being observed, and in the appropriate context, LUS can help to sort out patients to be discharged or admitted for further observation, without the need for a CT scan. This decision can be easily taken provided LUS is available in the ED, with the added advantage of avoiding a radiological approach. LUS will not tell a COVID-19 patient apart from others displaying the same interstitial features, although in the appropriate context, COVID-19 can be assumed and it accurately quantifies severity. Decisions that apply specifically to COVID-19 patients can only be implemented in the high-prevalence scenario.

The main interest of this study was to explore the capacity of LUS to quantify lung injury in COVID-19 pneumonia. We compared our score with a corresponding CT scan score, used as a gold standard. Zones A, B, and PLAPS on either side of the chest of the LUS examination were compared with topographic equivalents of the radiological examination. Scores for the whole lung were compared through correlation and agreement. We opted for a model of 6 views, although other authors prefer more extended examinations with up to 14 views[10] improved accuracy with these more extended approaches is to be demonstrated and, in our understanding, may compromise the intrinsic and distinguishing expeditiousness of the LUS examination.

We have found more B-line artifacts than consolidation. This may be because B lines are found in the early stages of the disease, whereas consolidation tends to appear later. This association has been explored in a study by de Almeida Monteiro et al[18] that opted for an original model to explore the capacity of LUS to detect patterns of the severity of lung injury in COVID-19 pneumonia, relating LUS findings to pathological specimens

obtained soon after death. Their comparison found a good correlation between subpleural and pulmonary consolidation and fibrosis, a link that could be a marker of gravity and prognosis in these patients; given the fact that fibrosis is related to a poorer outcome. On the other hand, although it is worth remembering the capacity of LUS to detect consolidation, pleural effusion, and heart failure, CT scans could probably outperform LUS when a wider differential diagnosis of respiratory conditions is entertained. Amongst the weaknesses of this study is the small number of patients investigated, although statistically significant agreement and correlation were disclosed between the methods. Also, it can be argued that the method employed to compare LUS and CT scans is new and that its reliability proper is at stake. To compensate for this objectionable feature, we most extensively explained how we constructed the scores and how they were matched. Furthermore, we acknowledged that our score is not always easily applicable. For instance, differences between confluent B lines and white lungs are not always easy to define. For the same reason, irregular and broken pleural lines are often a subtle difference. But we understand that these discriminations are often possible to establish and represent different levels of damage that should be explored. Finally, COVID-19 is no longer either a frequent or serious condition presenting at ED, although this model could be potentially applied to other conditions presenting as interstitial lung syndrome. We concluded that LUS, as compared to CT scans, is an effective method to estimate degrees of lung injury in patients with suspected COVID-19 in the ED.

Authorship

Luiz C. Pallarés and Ana P. Zanardo designed and implemented the study. Jonas M. Wolf performed the statistical analyses. Luiz C. Pallarés, Ana P. Zanardo, V. de Souza, and Jonas M. Wolf. Luiz C. Pallarés, Ana P. Zanardo, V. de Souza, and Jonas M. Wolf wrote the first draft of the manuscript and contributed to the literature review and discussion of the results. All authors contributed to and have approved the final manuscript.

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Conflicts of interest

The authors declare no conflicts of interest.

Data availability statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

REFERENCES

1. Saraogi A. Lung ultrasound: present and future. *Lung India*. 2015;32(3):250-7.
2. Rafailidis V, Apostolou D, Kaitartzis C, Rafailidis D. Ultrasonography of the healing process during a 3-month follow-up after a splenic injury. *Ultrasonography*. 2015;34(3):226-30.
3. Kory PD, Pellecchia CM, Shiloh AL, Mayo PH, DiBello C, Koenig S. Accuracy of ultrasonography performed by critical care physicians for the diagnosis of DVT. *Chest*. 2011;139(3):538-42.
4. Peterson, D, Arntfield RT. Critical care ultrasonography. *Emerg Med Clin North Am*. 2014;32(4):907-26.
5. Cascella M, Rajnik M, Aleem A, Dulebohn SC, Di Napoli R. Features, Evaluation, and Treatment of Coronavirus (COVID-19). 2022 Oct 13. *StatPearls* [Internet]. 2023 Aug [cited Mar 12]. Available from: https://www.ncbi.nlm.nih.gov/books/NBK554776/PMID: 32150360.
6. Lichtenstein DA. BLUE-protocol and FALLS-protocol: two applications of lung ultrasound in the critically ill. *Chest*. 2015;147(6):1659-70.
7. Lichtenstein DA, Mezière GA. Relevance of lung ultrasound in the diagnosis of acute respiratory failure: the BLUE protocol. *Chest*. 2008;134(1):117-25.
8. Lichtenstein D, Mézière G, Biderman P, Gepner A, Barré O. The comet-tail artifact. An ultrasound sign of alveolar-interstitial syndrome. *Am J Respir Crit Care Med*. 1997;156(5):1640-6.
9. Bland JM, Altman DG. Statistical methods for assessing agreement between two methods of clinical measurement. *Lancet*. 1986;1:307-10.
10. Allinovi M, Parise A, Giacalone M, Amerio A, Delsante M, Odone A, et al. Lung ultrasound may support diagnosis and monitoring of COVID-19 pneumonia. Ultrasound Med Biol. 2020;46(11):2908-17.
11. Islam N, Ebrahimzadeh S, Salameh JP, et al. Thoracic imaging tests for the diagnosis of COVID-19. *Cochrane Database Syst Rev*. 2021;3(3):CD013639.
12. Narinx N, Smismans A, Symons R, Frans J, Demeyere A, Gillis M. Feasibility of using point-of-care lung ultrasound for early triage of COVID-19 patients in the emergency room. *Emerg Radiol*. 2020;27(6):663-70.

13. Fonsi GB, Sapienza P, Brachini G, Andreoli C, De Cicco ML, Cirillo B, et al. Is lung ultrasound imaging a worthwhile procedure for severe acute respiratory syndrome coronavirus 2 pneumonia detection? *J Ultrasound Med*. 2021;40(6):1113-23.

14. Gil-Rodríguez J, Rojas JP, Aranda-Laserna P, Benavente-Fernández A, Martos-Ruiz M, Peregrina-Rivas JA, et al. Ultrasound findings of lung ultrasonography in COVID-19: a systematic review. *Eur J Radiol*. 2022;148:110156.

15. Zanforlin A, Strapazzon G, Falk M, Gallina V, Viteritti A, Valzolgher L, et al. Lung ultrasound in the emergency department for early identification of COVID-19 pneumonia. *Respiration*. 2021;100(2):145-53.

16. Patel R, Babady E, Theel ES, Storch GA, Pinsky BA, St George K, et al. Report from the American Society for Microbiology COVID-19 International Summit, 23 March 2020: value of diagnostic testing for SARS-CoV-2/COVID-19. *mBio*. 2020;11(2):e00722-20.

17. Trauer MM, Matthies A, Mani N, McDermott C, Jarman R. The utility of lung ultrasound in COVID-19: a systematic scoping review. *Ultrasound*. 2020;28(4):208-22.

18. Monteiro RAM, Duarte-Neto AN, Silva LFF, Oliveira EP, Nascimento ECT, Mauad T, et al. Ultrasound assessment of pulmonary fibroproliferative changes in severe COVID-19: a quantitative correlation study with histopathological findings. *Intensive Care Med*. 2021;47(2):199-207.

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Update of clinical aspects of knee osteoarthritis: data mining from previous work with NMR-based metabolomics of synovial fluid

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ABSTRACT

Introduction: Knee osteoarthritis (OA), which leads to progressive disability, has been associated with metabolic syndrome. Metabolomics emerges as a promising tool for investigation of this connection. The goal of this study was to correlate the metabolic profile of synovial fluid of patients with knee osteoarthritis with clinical factors related to the development of metabolic syndrome (MetS) based on the reanalysis of a patient's database from our previous study.

Methods: Patients were divided in two groups: without osteoarthritis, who underwent knee arthroscopy (n = 8; K-L Grade 0) and with knee OA, who underwent total knee arthroplasty surgery (n = 26, K-L Grades 3-4). From a database of synovial fluid metabolomic analysis by nuclear magnetic resonance, clinical data and collected from medical records, including age, sex, height and weight, and fasting blood glucose levels underwent multivariate analysis.

Results: Based on the metabolic profile, glycerol was increased in the group of patients with osteoarthritis. However, metabolomics was not able to classify patients into subgroups according to blood glucose ranges, body mass index and by age.

Conclusion: In a population with osteoarthritis, metabolic analysis evidences a slightly different profile in the analysis by age and BMI, or age and glycaemia.

Keywords: *Metabolomics; Metabolic Syndrome; Osteoarthritis; Synovial Fluid*

INTRODUCTION

Knee osteoarthritis (OA) is characterized by synovial inflammation, hyaline cartilage degeneration and subchondral bone thickening, affecting the whole joint[1]. Metabolic syndrome (MetS) is defined by the presence of three or more clinical findings of hyperglycemia, obesity, dyslipidemia, and systemic hypertension, hence related to increased rates of cardiovascular disease and mortality[2].

Obesity has long been associated to OA due to mechanical aspects of overweight, but it has also a role in consequence of low-grade inflammation leading to subchondral bone microvasculature alterations and neuromuscular damage[3]. Hypertension seems to have an independent role in OA genesis, compared to other MetS components, since it produces ischemic modifications which can compromise articular cartilage and subchondral bone, hypothetically due to atheroma plates formation[4,5]. Dyslipidemia promotes lipidic deposition in articular cartilage, seeming to damage its structure[5]. Insulin resistance, or lack of this hormone, hinders glucose uptake by cells, leading to an increase of its plasmatic concentration and impairing chondrocyte ability to maintain and repair extracellular matrix[6].

Metabolites constitute a broad range of low molecular weight (less than 1.5KD), which develop important roles in biological systems, allowing the understanding some diseases phenotypes[7]. Body fluids metabolites are influenced not only by genetics, but also by lifestyle, as diet, exercises, drug

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uptake, intestinal microbiome, hormonal homeostasis, and age[8]. Metabolomics is a large-scale analysis of metabolites and is central in flux of information[9]. There are two standard methods for metabolomics, NMR (Nuclear Magnetic Resonance) and MS (Mass Spectrometry). NMR has the advantage to be more reproducible and be indestructible for the sample. In addition, NMR allows in-vivo analysis, and minor liquid biofluid preparation. In the context of OA, NMR metabolomics seems to be a promising tool in finding an OA biomarker useful for diagnosis, stratification, and treatment control[10]. Our group described that OA is related to enhanced levels of glycerol and glucose in the synovial fluid[11].

The goal of this study was to correlate the synovial fluid metabolic profile of patients with and without knee osteoarthritis with clinical data related to metabolic syndrome.

MATERIAL AND METHODS

Study subjects

This study is a secondary analysis performed on clinical data of 34 patients included in a previous study[11] and approved by the Institutional Ethics Board.

The population study was composed of patients who underwent knee arthroscopy (without knee OA, K-L grade 0) or replacement (with knee OA, K-L grades III or IV), classified according to the American College of Rheumatology criteria of OA[12] and Kellgreen-Lawrence OA staging[13]. Patients with gout, pseudogout, rheumatoid arthritis, lupus, psoryasis, hemochromatosis, chronic inflammatory diseases, genetic diseases, autoimmune diseases, and cancer were not included in the study. Patients with infection and previous surgery on the affected knee were also not included, as so as the ones HIV positive, hepatitis B/C positive, in use of immunossuppresants, chemotherapy or corticosteroids. All patients signed the written informed consent.

Radiographic analysis for OA classification was based on the institutional image database and performed with the software mDicom Viewer 3.0.0 (Microdata).

Clinical data evaluation

Clinical data regarding age (in years), height (in meters) and weight (in kilograms) to calculate body mass index (BMI), and serum blood glucose (in milligrams per deciliter) were collected from medical records in the institutional preoperative evaluation form.

Regarding age, patients were stratified in two subgroups: inferior or equal to 70 years and superior to 70 years.

BMI was calculated by the ratio between weight, in kilograms, and height, in square meters, which is the most used method for calculating body adiposity. For BMI analysis we classified the patients according to the Brazilian Consensus on Obesity[14] in normal weight (18.5 to 24.9 kg/m^2), overweight (25 to 29.9 kg/m^2) and obese (≥ 30 kg/m^2).

Blood glucose is measured in serum after 8 to 12 hours fasting, without consumption of food or beverage, except water. In this study we used reference levels adapted from the Brazilian Consensus of Diabetes[15], classified as normal (< 100 mg/dL), prediabetes (100 to 125 mg/dL or diabetes (≥ 126 mg/dL).

Metabolic evaluation of synovial fluid

Synovial fluid harvest, transport and NMR metabolomics analysis from the database samples were described as performed in a previous study[11]. For the new analysis we only used the unsupervised principal component analysis (PCA). All the assignments were performed by COLMARm (https://spin.ccic.osu.edu/index.php/colmarm) with HSQC and TOCSY and 1D spectra by HMDB (https://hmdb.ca/) and BMRB (http://www.bmrb.wisc.edu/metabolomics/).

Metabolic analysis of subgroups first included isolated analysis of Metabolomics by age (below and above 70 years), BMI (normal, overweight, and obese), and blood glucose levels (normal, prediabetes and diabetes). Then, analysis focused only on patients with knee OA and the associations between age and BMI, and age and blood glucose levels, in order to find if there was any metabolite able to differentiate these groups.

Statistical analysis

Data were collected in Microsoft Excel® spreadsheets and statistical analysis was performed by the software GraphPad Prism for Windows 5.0.

Age, BMI, and blood glucose were presented in average and standard deviation and compared by unpaired one-tailed Student´s *t*-test. Chi-square test was used to compare proportion of patients with and without OA and subgroups of BMI and blood glucose.

The matrix with NMR analysis data was performed on AMIX software (Bruker). Multivariate statistical analyses were done on MetaboAnalyst 4.0 (www.metaboanalyst.ca) and normalized by the sum of peak intensities and pareto-scaling, besides multivariate principal component analysis.

Posteriorly, data from metabolomic analysis archives underwent multivariate analysis according to the clinical data obtained from medical records in order to identify metabolites that could discriminate those groups. Analysis involved patients with knee OA which presented age over 70 years and BMI equal or superior to 30 kg/m^2 versus the ones with age

equal or inferior to 70 years and BMI below 30 kg/m^2. In the same manner we investigated patients with knee OA that had age over 70 years and blood glucose levels equal or superior to 100 mg/dL versus the ones with age equal or inferior to 70 years and blood glucose levels below 100 mg/dL. *p* value was considered significant when below 0.05.

RESULTS

Demographics

Clinical profile evidenced that patients with knee OA (n = 26) presented higher age, BMI, and blood glucose levels average than the ones without knee OA (n = 8), as shown in Table 1.

Table 1: Demographic data of patients included in the study.

	W/OA (n=26)	W/O OA (n=8)	*p* value	95% CI
OA Classification	Grade 3: 4 Grade 4: 22	Grade 0: 8	N/A	N/A
Age	67.35 ± 0.97	26.75 ± 2.4	*p*<0.0001*	36.10 – 45.09
Sex	Male:4	Male: 8		
	Female:22	Female:0	N/A	N/A
BMI	32.75 ± 1.1	27.26 ± 1.07	*p*=0.0028**	1.20 – 9.76
Blood glucose	107.6 ± 4.09	85.63 ± 3.38	*p* =0.0401**	6.35 – 37.63

W/OA: with OA; W/O OA: without OA; n: number of patients; CI: confidence interval; BMI: body mass index; N/A: non-applicable; * one-tailed unpaired *t*-test; ** two-tailed unpaired *t*-test.

Clinical data and OA

Patients with and without knee OA were then stratified in subgroups according to the variables BMI (normal, overweight and, obese) and fasting blood glucose level (normal, prediabetes and diabetes).

A significant association between obesity and OA (p = 0.0061) was identified. It´s important to highlight that 7.7% patients with OA did not present abnormal BMI. However, no significant association between fasting blood glucose levels and OA was verified (p = 0.23).

Synovial fluid metabolic profile

To investigate if synovial fluid metabolic profile was related to presence of OA, age, BMI, or fasting blood glucose levels, registries from de Sousa et al study database (10) were re-evaluated by PCA (Principal Component Analysis) guided by clinical data.

Individuals were first classified in two distinct subgroups, without OA and with OA according to metabolic profile (Figure 1A). Original database analysis identified that the metabolite “glucose + glycerol”, chemical shift 3.56 ppm (Figure 1B) was the main responsible for allowing the two groups distinction, which was confirmed in this re-analysis. Based on this separation, we could proceed with further data analysis.

Since all OA patients were over 55 years old, we established a cut point of 70 years for metabolic analysis by age. However, PCA was not able to classify individuals in these two subgroups according to metabolic profile, as there was a clear intersection between the profiles in score plot (Figure 2A), and no assigned metabolite was able to distinguish patients group considering the clinical data for each section (Figure 2B).

PCA analysis evidenced that normal BMI patients presented slightly different synovial fluid metabolic profile than overweight and obese patients (Figure 2C), but no assigned metabolite was able to distinguish patients group considering the clinical data for each section (Figure 2D).

PCA analysis evidenced that normal glycaemia patients presented slightly different synovial fluid metabolic profile than prediabetic and diabetic patients (Figure 3A), but no assigned metabolite was able to distinguish patients group considering the clinical data for each section (Figure 3B).

Clinical data from OA patients were combined to verify if the association of the variables age, BMI, and blood glucose levels could help identifying different metabolic profiles.

PCA analysis evidenced, in Score Plot (Figure 4A), that patients from subgroup A (BMI < 30 kg/m^2 and age ≤ 70 years) presented slightly different synovial fluid metabolic profile than patients in subgroup B (BMI ≥ 30 kg/m^2 and age > 70 years), but no assigned metabolite was able to distinguish patients group considering the clinical data for each section (Figure 4B).

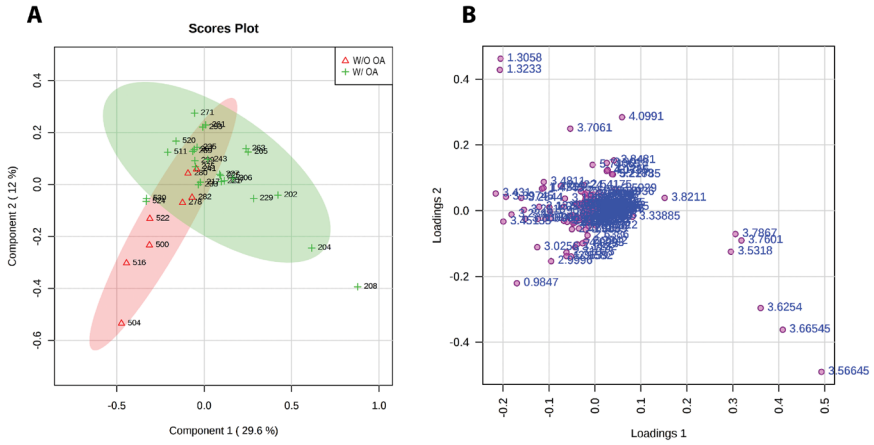

Figure 1: Discrimination of patients without and with OA by metabolic profile. PCA evidenced that it was possible to distinguish patients without OA (red diagram) from those with OA (green diagram) by metabolic profile. A: Score-Plot, PC 2 versus PC 1, where each dot represents one patient. Percentage in parenthesis evidence that PC1 can explain 29.6% of variables, and PC2 only 12%; B: Loading-Plot, where each dot represents one bucket, indicated that the metabolite "glucose + glycerol", chemical dislocation 3.56 ppm was the main responsible for this distinction. OA: Osteoarthritis; PCA: Principal component analysis; ppm: parts per million.

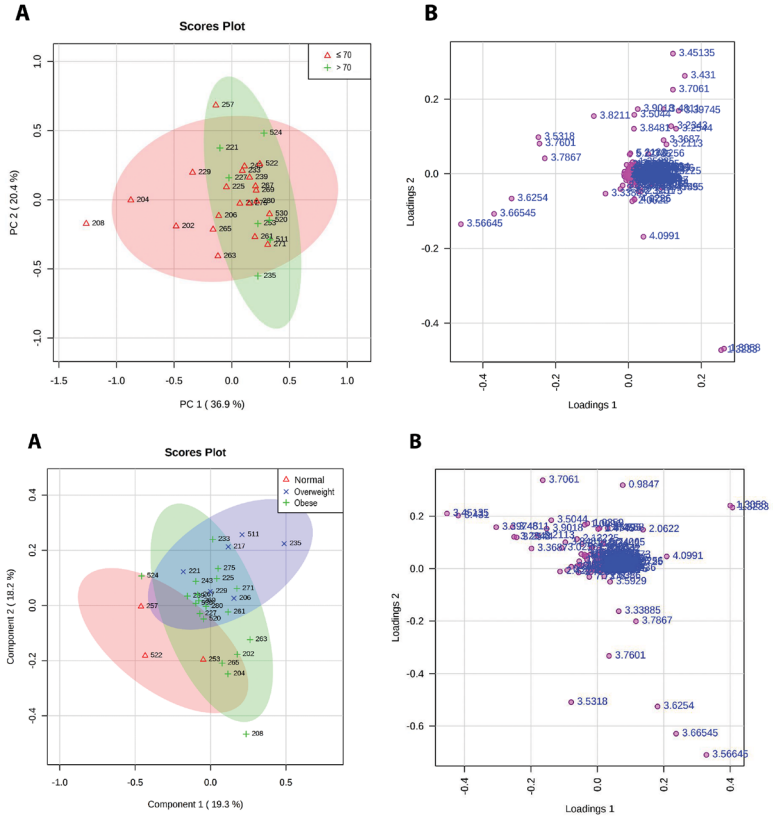

Figure 2: Discrimination of patients according to age and BMI by metabolic profile. A: PCA evidenced that it was not possible to distinguish patients with age equal or below 70 years (red diagram) from those with age above 70 years (green diagram) by metabolic profile B; Loading-Plot, where each dot represents one metabolite, indicated that no assigned metabolite was able to distinguish patients group considering the clinical data for each section. OA: osteoarthritis; PCA: principal component analysis. C: PCA analysis evidenced that normal BMI patients (red diagram) presented slightly different metabolic profile than overweight (blue diagram) and obese patients (green diagram); D: Loading-Plot, where each dot represents one metabolite, indicated that no assigned metabolite was able to distinguish patients group considering the clinical data for each section. OA: osteoarthritis; PCA: principal component analysis.

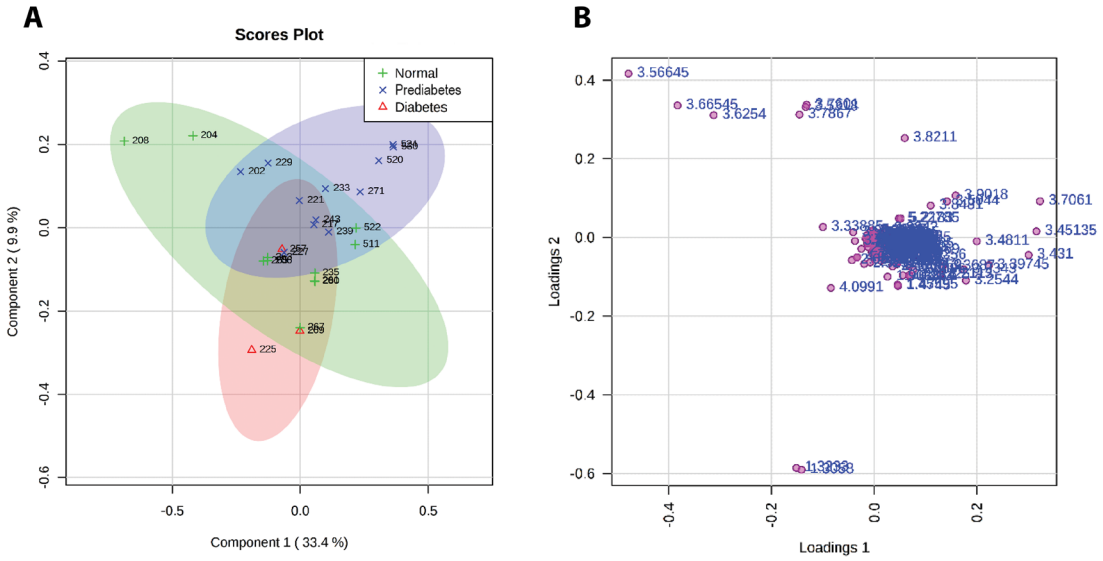

Figure 3: Discrimination of patients according to fasting blood glucose level by metabolic profile. PCA analysis pointed to convergence between normal fasting glycemia (green diagram), prediabetic (blue diagram) and diabetic (red diagram) patients, which impaired their distinction by metabolic profile. A: Score-Plot, PC 2 versus PC 1, where each dot represents one patient. Percentage in parenthesis evidence that PC1 can explain 33.4% of variables, and PC2 only 9.9%; B: Loading-Plot, where each dot represents one metabolite, indicated that no assigned metabolite was able to distinguish patients group considering the clinical data for each section. OA: osteoarthritis; PCA: principal component analysis.

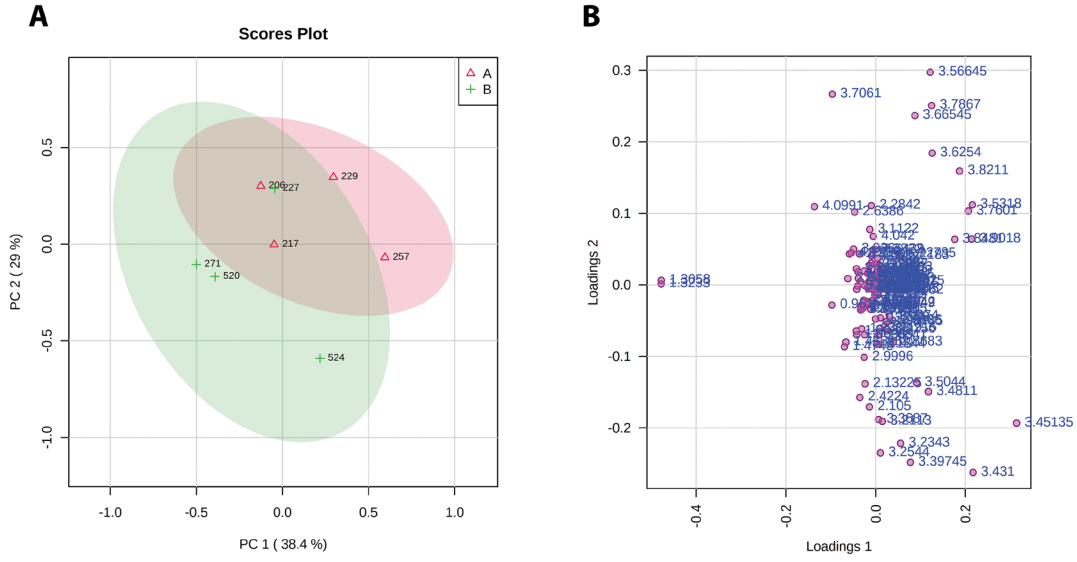

Figure 4: Discrimination of OA patients according to the association between age and BMI by metabolic profile. PCA analysis evidenced that patients in subgroup A (BMI < 30 kg/m2 and age ≤ 70 years) presented slightly different synovial fluid metabolic profile than patients in subgroup B (BMI ≥ 30 kg/m2 and age > 70 years). A: Score-Plot, PC 2 versus PC 1, where each dot represents one patient. Percentage in parenthesis evidences that PC1 can explain 38.4% of variables, and PC2 only 29%; B: Loading-Plot, where each dot represents one metabolite, indicated that no assigned metabolite was able to distinguish patients group considering the clinical data for each section. OA: osteoarthritis; BMI: body mass index; PCA: principal component analysis.

Similarly, PCA analysis evidenced, in Score Plot (Figure 5A), that patients from subgroup C (blood glucose < 100 mg/dL and age ≤ 70 years) presented slightly different synovial fluid metabolic profile than patients in subgroup D (blood glucose ≥ 100 mg/dL and age > 70 years), but no assigned metabolite was able to distinguish patients group considering the clinical data for each section (Figure 5B).

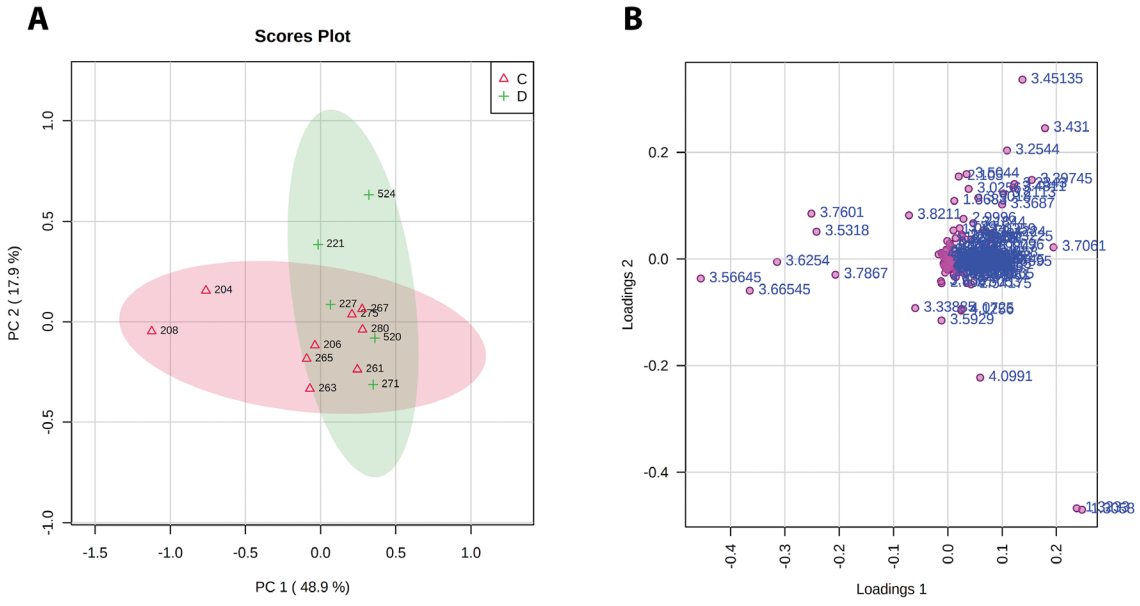

Figure 5: Discrimination of OA patients according to the association between age and fasting blood glucose level by metabolic profile. PCA analysis evidenced that patients in subgroup C (blood glucose < 100 mg/dL and age ≤ 70 years) presented slightly different synovial fluid metabolic profile than patients in subgroup D (blood glucose ≥ 100 mg/dL and age > 70 years). A: Score-Plot, PC 2 versus PC 1, where each dot represents one patient. Percentage in parenthesis evidences that PC1 can explain 48.9% of variables, and PC2 only 17.9%; B: Loading-Plot, where each dot represents one metabolite, indicated that no assigned metabolite was able to distinguish patients group considering the clinical data for each section. OA: osteoarthritis; BMI: body mass index; PCA: principal component analysis.

DISCUSSION

Osteoarthritis (OA) is a chronic complex degenerative disease characterized by cartilage degradation, synovial inflammation, and bone thickening, besides osteophytes formation, affecting the synovial joint as a whole and leading to loss of articular function[16,17].

In this research we performed an analysis of medical records from a database of patients included in a previous study[11] and evaluated their relationship with synovial fluid metabolomic analysis. We confirmed the previous findings that glucose and glycerol were able to classify patients without and with knee OA. However, this classification according to the metabolic profile was not possible neither when considered age, BMI, and fasting blood glucose levels, nor when associating the variables age and BMI, or age and fasting blood glucose levels. In this study, an analysis using principal component analysis (PCA), simpler, was performed, since it was a database re-evaluation, under new parameters, stratified in classes, not performed before. Besides, PCA is less susceptible to data over fitting, permitting visualization without complex statistical analysis.

Synovial fluid is a plasma ultrafiltrate, containing additional molecules produced by cells in joint space, reflecting its biological phenomena. Besides, it is in contact with tissues affected by knee OA, as so as synovial fluid may represent articular tissues alterations more precisely than measurements made in blood or urine[18,19].

In this study, we identified that clinical variables as glycaemia and obesity, as so as age, were not able to distinguish different patient profiles through PCA since this is an unsupervised analysis and, possibly, due to the great variability found in the samples, which can be explained by diet, genetics and ancestry of patients analyzed. Age, sex, BMI, and comorbidities have already been described as possible confusing factors in knee OA patient's synovial fluid metabolic

analysis. However, in a previous study, no metabolic profile related to these factors was found[8].

We chose 70 years old as cut point for age due to the profile of our OA patients include only the ones who underwent total knee replacement, and hence be aged above 55 years old. As knee OA incidence and severity increase with age[17], we thought that the age of 70 years old would be able to identify patients with metabolic profile compatible with later stages of the disease. The age of 50 years old was used as cut point previously in an attempt to create a predictive model of metabolite signatures for knee OA diagnosis, suggesting that phosphatidylcholine and lysophosphatidylcholine would be the predominant indicators of knee OA in older males[20]. Neither alteration in phosphatidylcholine concentrations was found. It had been previously demonstrated that many metabolites concentration is affected by sex, race or age, which is considered preponderant. Hence, those factors may be confounding factors when different groups are compared in clinical studies[21].

Metabolic syndrome, defined by the presence of systemic hypertension, dyslipidemia, hyperglycemia and obesity, seems to increase knee OA severity, notably in relation to the worsening of clinical manifestations and prognosis, due to cumulative influence of metabolic disorders, in a chronic state of low-grade inflammation[2,16,22]. It was suggested that alterations in amino acids metabolism seems to have a central role in MetS[23]. However, although its clinical relevance to public health, a recent systematic review evidenced that few papers were published focusing on MetS biomarkers investigation[24].

In this study, we chose BMI and fasting blood glucose levels, available in institutional medical records of patients, as clinical parameters for stratification analysis of metabolic profile. Analysis stratified by BMI evidenced that normal BMI patients presented slightly different synovial fluid metabolic profile than overweight and obese patients. However, this difference was not statistically significant. It has been reported that other studies tried to stratify patients by BMI, in normal and obese, according to the metabolic profile, using analysis as PCA. However, confounding factors as diet, socioeconomic status, physical activity, tabagism and sex, among others, turn this analysis harder[25].

Knee OA and obesity relationships were investigated by untargeted serum metabolomic analysis and evidenced that obesity related knee OA presents greater oxidative stress and more acid medium. Besides, oxidative stress based on nitrogen reactive species and acidotic problems are increasing in obesity in contrast to non-obesity related knee OA[26]. Metabolic profiles that could distinguish overweight and obese adults presenting progressive and non-progressive signs of radiographic OA were identified, suggesting a role of metabolic factors in disease evolution[27]. In turn, it was also revealed that OA patient's metabolic profile alters robustly over radiographic staging of the disease, with greater concentrations of malate and a tendency to increased concentrations of citrate, fumarate, and succinate in late stages of the disease. These findings suggest that metabolism in OA late stages converges to preserve energy[28]. Our data evidenced a slightly different metabolic profile of normal from abnormal BMI patients, but it did not allow significant distinction of neither of all patients by BMI, nor of OA patients by age and BMI based on synovial fluid metabolic profile.

Regarding diabetes, there are evidences that glucose, fructose, amino acids and lipids are altered both in patients with type I and II diabetes[29]. Similarly, recent study identified 12 plasma metabolites, by mass spectroscopy, in patients without medical records of diabetes (48.1% men, average age 63.3 years), which could be related to different glucose metabolism indexes and insulin sensitivity compared to general population[30]. In the present study, analysis based on fasting glycaemia indicated that normal glycaemia patients presented slightly similar metabolic profile to prediabetic and diabetic patients, although no metabolite could identify this classification. We believe the fact that our analysis been performed in synovial fluid and not on plasma may be one of the factors responsible for this difference in findings between the studies.

Studies pointed out the relationship between type 2 diabetes and knee OA progression in any joint and, specially, with two times chances of association with knee replacement than in normal population[31]. Another study demonstrated that glucose homeostasis can predict individuals at risk for OA development, but are not able to distinguish cases of accelerated knee OA[32]. This is corroborated by our data, which evidenced a slightly different metabolic profile of normal glycaemia from prediabetic and diabetic patients, but it did not allow significant distinction of neither of all patients by blood glucose levels, nor of OA patients by age and blood glucose levels based on synovial fluid metabolic profile.

Finally, we identified that although patients presented different OA grades, age, BMI, and glycaemia, they could only be classified by OA absence/presence, according to metabolic profile.

This study presents some limitations, as the small sample size and incomplete medical records, which turned it difficult to represent all possibilities of profiles. Moreover, lipidogram was not available in medical records, and so it was not used for subgroup stratification for analysis, although dyslipidemia is

one of the components of MetS. However, we could analyze synovial fluid from live patients without and with knee OA, instead of some studies involving cadavers or only diseased samples.

In conclusion, metabolomics was able to classify patients without and with knee OA according to metabolic profile, due to increased glycerol and glucose concentration in the OA subgroup. However, considering only the OA patients, metabolomics evidenced a slightly different metabolic profile according to the associations between age and BMI, or age and glycaemia, which point out to a possible association between MetS with OA.

Conflict of interests

The authors have no conflict of interests to declare.

REFERENCES

1. Goldring MB, Goldring SR. Osteoarthritis. *J Cell Physiol*. 2007;213(3):626-34.
2. Zhuo Q, Yang W, Chen J, Wang Y. Metabolic syndrome meets osteoarthritis. *Nat Rev Rheumatol*. 2012;8(12):729-37.
3. Niu J, Clancy M, Aliabadi P, Vasan R, Felson DT. Metabolic syndrome, its components, and knee osteoarthritis: the framingham osteoarthritis study. *Arthritis Rheumatol*. 2017;69(6):1194-1203.
4. Chan PB, Wen C. Spontaneous hypertensive rat exhibits bone and meniscus phenotypes of osteoarthritis: Is it an appropriate control for MetS-associated OA? *Ann Rheum Dis*. 2018;77(5):e25.
5. Walter SS, Wintermeyer E, Klinger C, Lorbeer R, Rathmann W, Peters A, et al. Association between metabolic syndrome and hip osteoarthritis in middle-aged men and women from the general population. *PLoS One*. 2020;15(3):e0230185.
6. Zhai G. Alteration of metabolic pathways in osteoarthritis. *Metabolites*. 2019;9(1):11.
7. Zhang W, Likhodii S, Zhang Y, Aref-Eshghi E, Harper PE, Randell E, et al. Classification of osteoarthritis phenotypes by metabolomics analysis. *BMJ Open*. 2014;4(11):e006286.
8. Guma M, Tiziani S, Firestein GS. Metabolomics in rheumatic diseases: desperately seeking biomarkers. *Nat Rev Rheumatol*. 2016;12(5):269-81.
9. Santos Jr GCS, Renovato-Martins M, Brito NM. The remodel of the "central dogma": a metabolomics interaction perspective. Metabolomics. 2021;17(48):1-15.
10. Sousa EB, Santos Junior GC, Duarte MEL, Moura Neto V, Aguiar DP. Metabolomics as a promising tool for early osteoarthritis diagnosis. *Braz J Med Biol Res*. 2017;50(11).
11. Sousa EB, Santos Junior GC, Aguiar RP, Sartore RC, Oliveira ACL, Almeida FCL, et al. Osteoarthritic synovial fluid modulates cell phenotype and metabolic behavior *in vitro*. *Stem Cells Int*. 2019;15(2019):8169172.
12. Altman R, Asch E, Bloch D, Bole G, Borenstein D, Brandt K, et al. Development of criteria for the classification and reporting of osteoarthritis: Classification of osteoarthritis of the knee. Diagnostic and Therapeutic Criteria Committee of the American Rheumatism Association. *Arthritis Rheum*. 1986;29(8):1039-49.
13. Kellgren JH, Lawrence JS. Radiological assessment of osteo-arthrosis. *Ann Rheum Dis*. 1957;16(4):494-502.
14. Associação Brasileiro para o Estudo da Obesidade e Síndrome Metabólica. *Diretrizes brasileiras de obesidade*. São Paulo: ABESO; 2016.
15. Sociedade Brasileira de Diabetes. *Consenso Brasileiro de Diabetes, Sociedade Brasileira de Diabetes Diretrizes 2017-2018*. São Paulo: Sociedade Brasileira de Diabetes; 2017.
16. Berenbaum F. Deep phenotyping of osteoarthritis: a step forward. *Ann Rheum Dis*. 2019;78(1):3-5.
17. Munjal A, Bapat S, Hubbard D, Hunter M, Kolhe R, Fulzele S. Advances in molecular biomarker for early diagnosis of osteoarthritis. *Biomol Concepts*. 2019;10(1):111-9.
18. Carlson AK, Rawle RA, Wallace CW, Brooks EG, Adams E, Greenwood MC, et al. Characterization of synovial fluid metabolomic phenotypes of cartilage morphological changes associated with osteoarthritis. *Osteoarthritis Cartilage*. 2019;27(8):1174-84.
19. Watt FE, Hamid B, Garriga C, Judge A, Hrusecka R, Custers RJH, et al. The molecular profile of synovial fluid changes upon joint distraction and is associated with clinical response in knee osteoarthritis. *Osteoarthritis Cartilage*. 2020;28(3):324-33.
20. Rockel JS, Kapoor M. The metabolome and osteoarthritis: Possible contributions to symptoms and pathology. *Metabolites*. 2018;8(4):92.
21. Lawton KA, Berger A, Mitchell M, Milgram KE, Evans AM, Guo L, et al. Analysis of the adult human plasma metabolome. *Pharmacogenomics*. 2008;9(4):383-97.
22. Dickson BM, Roelofs AJ, Rochford JJ, Wilson HM, De Bari C. The burden of metabolic syndrome on osteoarthritic joints. *Arthritis Res Ther*. 2019;21(1):289.
23. Roberts JA, Varma VR, Huang CW, An Y, Oommen A, Tanaka T, et al. Blood Metabolite Signature of Metabolic Syndrome Implicates Alterations in Amino Acid Metabolism: Findings from the Baltimore Longitudinal Study of Aging (BLSA) and the Tsuruoka Metabolomics Cohort Study (TMCS). *Int J Mol Sci*. 2020;21(4):1249.
24. Monnerie S, Comte B, Ziegler D, Morais JA, Pujos-Guillot E, Gaudreau P. Metabolomic and lipidomic signatures of metabolic syndrome and its physiological components in adults: a systematic review. *Sci Rep*. 2020;10(1):669.
25. Rauschert S, Kirchberg FF, Marchioro L, Koletzko B, Hellmuth C, Uhl O. Early programming of obesity throughout

the life course: a metabolomics perspective. *Ann Nutr Metab*. 2017;70(3):201-9.

26. Senol O, Gundogdu G, Gundogdu K, Miloglu FD. Investigation of the relationships between knee osteoarthritis and obesity via untargeted metabolomics analysis. *Clin Rheumatol*. 2019;38(5):1351-60.

27. Loeser RF, Goldring SR, Scanzello CR, Goldring MB. Osteoarthritis: a disease of the joint as an organ. *Arthritis Rheum*. 2012;64(6):1697-707.

28. Kim S, Hwang J, Kim J, Ahn JK, Cha HS, Kim KH. Metabolite profiles of synovial fluid change with the radiographic severity of knee osteoarthritis. *Joint Bone Spine*. 2017;84(5):605-10.

29. Arneth B, Arneth R, Shams M. Metabolomics of type 1 and type 2 diabetes. *Int J Mol Sci*. 2019;20(10):2467.

30. Bos MM, Noordam R, Bennett K, Beekman M, Mook-Kanamori DO, van Dijk KW, et al. Metabolomics analyses in non-diabetic middle-aged individuals reveal metabolites impacting early glucose disturbances and insulin sensitivity. *Metabolomics*. 2020;16(3):35.

31. Courties A, Berenbaum F, Sellam J. The phenotypic approach to osteoarthritis: a look at metabolic syndrome-associated osteoarthritis. *Joint Bone Spine*. 2019;86(6):725-30.

32. Driban JB, Eaton CB, Amin M, Stout AC, Price LL, Lu B, et al. Glucose homeostasis influences the risk of incident knee osteoarthritis: data from the osteoarthritis initiative. *J Orthop Res*. 2017;35(10):2282-87.

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Perfil epidemiológico dos pacientes atendidos em programa de acompanhamento e tratamento da osteoartrite do joelho: estudo observacional e transversal

Epidemiological profile of patients undergoing a program for non-operative treatment of knee osteoarthritis: observational and sectional study

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RESUMO

Introdução: A osteoartrite (OA) é principal causa de diminuição da função articular, com impacto nas atividades da vida diária. O objetivo do estudo foi avaliar o perfil epidemiológico dos pacientes atendidos em um ambulatório de tratamento conservador da osteoartrite do joelho em uma instituição de nível terciário do Sistema Único de Saúde.

Métodos: Foi realizado um estudo observacional e transversal avaliando 577 pacientes com osteoartrite do joelho submetidos a tratamento farmacológico, no período de setembro de 2014 a abril de 2019. Foram coletados os seguintes dados epidemiológicos: sexo; data de nascimento; peso e altura; lateralidade; gravidade da OA, pela classificação de Ahlback, modificada por Keyes e Goodfellow; presença de crepitação articular e desvio do eixo dos membros inferiores.

Resultados: Não foi observada diferença entre os grupos em relação a gravidade da OA (p = 0,75), sexo (p=0,90), presença de crepitação articular (p=0,57), lateralidade (p=0,89), desvio do eixo dos membros inferiores (p=0,75), nem histórico de tratamentos prévios (p=0,46).

Conclusão: O perfil da população de pacientes com osteoartrite do joelho submetidos ao tratamento conservador, em sua maioria, é do sexo feminino, com obesidade e idade superior a 60 anos. Os graus II e III de Ahlback são preponderantes.

Palavras-chave: epidemiologia; joelho; osteoartrite; tratamento conservador.

ABSTRACT

Introduction: Osteoarthritis (OA) is the main cause of joint function impairment, impacting daily life activities. The goal of this study was to evaluate the epidemiological profile of patients treated in an outpatient unit specialized in non-operative OA treatment in a tertiary public institution.

Methods: A cross-sectional study was performed evaluating 577 patients with knee OA under pharmacological treatment between September 2014 and April 2019. The following epidemiological data were collected from medical records: sex, height and weight (to calculate body mass index), laterality, OA severity according to Ahlback classification modified by Keyes and Goodfellow, presence of articular crepitus and lower limb malalignment.

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Results: No difference was observed between the two groups regarding OA severity (p = 0.75), distribution by gender (p=0.90), presence of joint crepitus (p=0.57), laterality of OA involvement (p=0.89), presence of axis deviation of the lower limbs (p=0.75), nor history of previous treatments (p=0.46).

Conclusion: We verified that the population of patients with knee OA under non-operative treatment is in its majority female, obese and age over 60 years old. Alhback grades I and III are preponderant.

Keywords: epidemiology, knee; non-operative treatment; osteoarthritis.

INTRODUÇÃO

A osteoartrite (OA) é a principal causa da diminuição da função articular observada em todo o mundo, afetando cerca de 250 milhões de pessoas[1]. Possui origem multifatorial, sendo definida como uma degeneração articular progressiva, com inflamação do tecido sinovial e destruição da cartilagem articular, acompanhadas por remodelamento do osso subcondral[2]. A fisiopatologia da OA continua desconhecida e envolve fatores genéticos e ambientais[3].

A articulação do joelho é complexa sob o ponto de vista funcional e, devido a sua transmissão de peso e suporte de cargas, é uma das articulações mais acometidas pela osteoartrite[4]. Isto é, a osteoartrite do joelho afeta pelo menos 30% da população com idade acima de 50 anos e 80% dos acima de 75 anos[5,6].

No Brasil, os estudos de prevalência são escassos, limitando a avaliação da doença no país tendo sido descrito que o número de casos quase dobrou de 2000 para 2017, evoluindo da 14ª para a 12ª causa de incapacidade naqueles de 50 a 69 anos[7].

O objetivo do estudo foi avaliar o perfil epidemiológico e clínico dos pacientes atendidos em um ambulatório exclusivo de tratamento conservador da osteoartrite do joelho em uma instituição de nível terciário do Sistema Único de Saúde.

MATERIAL E MÉTODOS

Trata-se de um estudo observacional e transversal. A população do estudo foi composta por pacientes com osteoartrite do joelho submetidos a tratamento conservador farmacológico no ambulatório do "Programa de Acompanhamento e Tratamento da Osteoartrite" do joelho no período de setembro de 2014 a abril de 2019. O estudo foi aprovado pelo Comitê de Ética em Pesquisa (CAAE n° 05374818.9.0000.5273, em 25/01/2019 e CAAE n° 35488920.7.0000.5273, em 30/10/2020).

Foram incluídos no estudo pacientes com osteoartrite primária que receberam condroprotetores orais e/ou que foram submetidos à viscossuplementação com ácido hialurônico fornecidos pela instituição. Foram excluídos os pacientes que apresentavam registros incompletos no prontuário e as duplicatas, isto é, pacientes que receberam medicação ou infiltração com ácido hialurônico em mais de uma ocasião.

A análise iniciou pela busca nos sistemas informatizados do setor de farmácia e ambulatório, conforme os critérios de inclusão. Foi então realizado o cruzamento das listagens para exclusão dos pacientes com consultas repetidas. A partir dessa listagem, os prontuários foram selecionados e os dados de interesse foram coletados através da análise dos prontuários: Sexo (masculino/feminino); Data de nascimento, para cálculo da idade; Peso (Kg) e altura (m), para cálculo do índice de massa corporal (Kg/m^2); classificação da obesidade de acordo com as diretrizes brasileiras de obesidade[8] (normal: 20-24,9; sobrepeso: 25-29,9; obesidade grau I: 30-34,9; obesidade grau II: 35-39,9; obesidade grau III: > 40); lateralidade de acometimento da OA (direito / esquerdo / bilateral); gravidade da OA, pela classificação de Ahlback, modificada por Keyes e Goodfellow; presença de crepitação articular (difusa / femoro-patelar / ausente); presença de desvios do eixo dos membros inferiores (varo / valgo / neutro). Foram então excluídos os pacientes com dados incompletos.

Os dados foram tabulados em planilha Microsoft Excel e analisados. Inicialmente foram submetidos à estatística descritiva e apresentados na forma de frequência relativa. Já a estatística analítica comparou os dados de idade, peso e altura através da ANOVA one-way, com teste de Bonferroni *post-hoc*. Já os dados de sexo, lateralidade, gravidade da OA, presença de crepitação articular e de desvio do eixo dos membros foram comparados utilizando o teste do Qui-quadrado. Consideramos significativas as comparações nas quais o valor de *p* foi inferior a 0,05.

RESULTADOS

O cruzamento dos dados do sistema de agendamento de consultas e do sistema de fornecimento de medicamentos pela unidade de farmácia resultou em uma população de 1575 pacientes no período de setembro de 2014 a maio de 2019. Após remoção daqueles que se enquadravam nos critérios de exclusão, pacientes com mais de um atendimento ou prontuários incompletos, chegou-se a um total de 577 pacientes que foram

incluídos na análise, sendo 250 que haviam sido submetidos ao tratamento oral e 327 ao tratamento com viscossuplementação.

A Figura 1 apresenta o fluxograma dos pacientes incluídos e excluídos do estudo.

A Tabela 1 apresenta o perfil dos pacientes que foram incluídos no estudo. Posteriormente, foram divididos conforme a gravidade da osteoartrite, segundo a classificação de Ahlback, subdividida em 5 graus, conforme apresentado na Tabela 2. Para a análise, uma vez que o grupo de pacientes com OA grau V de Ahlback apresentava tamanho pequeno de integrantes, foi optado pela análise conjunta com os pacientes OA grau IV.

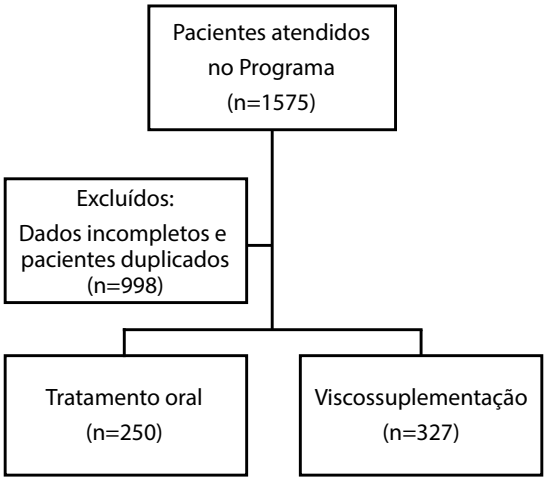

Figura 1: Fluxograma dos pacientes incluídos no estudo. No período selecionado, 1575 pacientes foram atendidos pelo programa. Após análise dos prontuários, verificou-se que 998 pacientes apresentavam dados incompletos ou haviam sido atendidos mais de uma vez. Desta forma, restaram 577 pacientes que foram incluídos no estudo e analisados de acordo com o tipo de tratamento recebido: tratamento oral (n = 250) ou viscossuplementação (n= 327). (n = número de pacientes.)

Tabela 1: Perfil dos pacientes incluídos no estudo. Dados absolutos de idade, peso e altura apresentados em média e desvio padrão. Demais dados apresentados em frequência absoluta e frequência relativa.

Variável		n= 577 (%)
Sexo	Masculino	190 (32,9)
	Feminino	387 (67,1)
Idade		62,44 ± 8,9
Peso (kg)		84,39 ± 16,69
Altura (m)		1,64 ± 0,1
IMC (kg/m^2)		31,25 ± 5,71
Obesidade	Normal	58 (10,1)
	Sobrepeso	199 (34,5)
	Obesidade grau I	187 (32,4)
	Obesidade grau II	95 (16,5)
	Obesidade grau III	38 (6,5)
Gravidade da OA	Ahlback I	70 (12,1)
	Ahlback II	286 (49,6)
	Ahlback III	167 (28,9)
	Ahlback IV+V	54 (9,3)
Crepitação	Difusa	194 (33,6)
	Femoro-Patelar	293 (50,8)
	Ausente	90 (15,6)
Lateralidade	Direito	321 (55,6)
	Esquerdo	256 (44,4)

Continua...

Tabela 1: Continuação.

Variável		n= 577 (%)
Eixo dos MMII	Varo	222 (38,5)
	Valgo	252 (43,7)
	Neutro	103 (17,9)
Tratamento Prévio	Sim	276 (47,8)
	Não	301 (52,2)
Tratamento Realizado	Tratamento Oral	250 (43,3)
	Viscossuplementação	327 (56,7)

Tabela 2: Distribuição dos pacientes incluídos no estudo e que realizaram tratamento conservador com medicação oral ou viscossuplementação, de acordo com a gravidade da osteoartrite. Dados absolutos de peso, idade e altura apresentados em média e desvio padrão. Demais dados apresentados em frequência relativa. **p* valor de acordo com o teste de Kruskal-Wallis (dados não-normais após análise pelo Teste de Pearson); ***p* valor de acordo com o teste do Qui-quadrado.

Características da amostra (n=577)		Gravidade da osteoartrite				*p valor*
		I	II	III	IV+V	
Sexo	Masculino	3.1 (25,7)	16.8 (33,9)	9.9 (34,1)	3.1 (33,3)	
	Feminino	9.0 (74,3)	32.8 (66,1)	19.1 (65,9)	6.2 (66,7)	0,59*
Idade		62,22 ± 8,1	61,92± 9,2	63,06 ± 8,5	63,37 ± 9,6	0,44**
Peso		81,85±16,5	84,08±15,6	84,79±17,7	88,14±17,7	0,23**
Altura		1,63±0,1	1,64±0,1	1,64±0,1	1,63±0,1	0,29**
IMC (kg/m^2)		30,1±5,1	30,9±5,5	31,6±5,8	32,3±6,6	0,21**
IMC	Normal	1,4 (11,4)	4,7 (9,4)	3,5 (12,0)	0,5 (5,6)	
	Sobrepeso	4,2 (34,3)	18,5 (37,4)	9,0 (31,1)	2,8 (29,6)	
	Obesidade grau I	4,0 (32,9)	14,9 (30,1)	9,7 (33,5)	3,8 (40,7)	
	Obesidade grau II	1,9 (15,7)	8,8 (17,8)	4,7 (16,2)	1,0 (11,1)	
	Obesidade grau III	0,7 (5,7)	2,6 (5,2)	2,1 (7,2)	1,2 (13,0)	0,54*
Crepitação	Difusa	2,9 (24,3)	24,3 (49.0)	18,0 (62,3)	8,5 (90,7)	
	Fêmuro-Patelar	6,6 (54,3)	21,5 (43.4)	9,9 (34,1)	0,9 (9,3)	
	Ausente	2,6 (21,4)	3,8 (7,7)	1,0 (3,6)	0 (0)	**<0,0001***
Lateralidade	Direito	7,3 (60,0)	27,6 (55,6)	15,1 (52,1)	5,7 (61,1)	
	Esquerdo	4,9 (40,0)	22.0 (44,4)	13.9 (47,9)	3,6 (38,9)	0,56*
Eixo dos MMII	Varo	3,3 (27,1)	19,4 (39,2)	14,9 (51,5)	6,2 (66,7)	
	Valgo	2,4 (20,0)	8,5 (17,1)	6,2 (21,6)	0,5 (5,6)	
	Neutro	6,4 (52,9)	21,7 (43,7)	7,8 (26,9)	2,6 (27,8)	**<0,0001***
Tratamento Prévio ou Associado	Sim	4,5 (37,1)	23,9 (48,3)	14,4 (49,7)	5,0 (53,7)	
	Não	7,6 (62,9)	25,6 (51,7)	14,6 (50,3)	4,3 (46,3)	0,24*
Tratamento utilizado	Tratamento Oral	5,9 (48,6)	20,3 (40,9)	12,5 (43,1)	4,7 (50,0)	
	Viscossuplementação	6,2 (51,4)	29,3 (59,1)	16,5 (56,9)	4,7 (50,0)	0,48*

DISCUSSÃO

Os pacientes com osteoartrite possuem um padrão epidemiológico parecido em todo o mundo, sendo mais prevalente no sexo feminino e com idade superior a 65 anos. O quadril e o joelho são as principais articulações grandes afetadas pela osteoartrite[1,9]. Nos EUA, a prevalência de osteoartrite é de 34% em pacientes com mais de 65 anos, sendo considerada sintomática em 16% da população com mais de 45 anos[10]. A doença atinge aproximadamente 19% das mulheres e 14% dos homens nesta mesma faixa etária[11]. No Brasil, apesar dos dados epidemiológicos em relação à doença serem escassos, tem se verificado um aumento no percentual de indivíduos acometidos pela doença nas últimas décadas, o que é compatível com a transição demográfica que ocorre no país[7].

Verificamos que 67,1% da nossa população atendida eram do sexo feminino, variando dentro da faixa de outros estudos com valores entre 49%[12], 52%[13], 55,8%[14], 60, 6%[15] e 69,9%[16]. Assim como verificado por Reginato et al[17], no nosso estudo o sexo feminino predominou em todos os grupos estudados, o que pode ser explicado pelas características demográficas da população brasileira. Segundo o censo de 2011, as mulheres representavam 55,8% da população brasileira acima de 60 anos e também apresentam expectativa de vida ao redor de 77 anos, superior à dos homens[18]. Além disso, ocorre menor procura dos homens pelos

serviços de atenção primária à saúde[19], justificando a sua menor presença no nível terciário de atenção, como é o caso do ambulatório usado como campo de estudo.

Em relação à idade, verificamos que a população do nosso estudo apresentou-se na faixa de 62,44 ± 8,9 anos, valor bem próximo aos encontrado em outros estudos por Reginato et al[17] (62,5 ± 10,5 anos), Lombnaes et al[16] (63,9 ± 8,9 anos) e Dorais et al[15] (68,9 anos). Identificamos ainda que os pacientes com osteoartrite grau II apresentavam média de idade inferior aos pacientes de graus III e IV + V, o que pode ser atribuído à própria história natural da doença, uma vez que pacientes mais idosos tendem a apresentar maior gravidade de destruição articular. Em contraste, os pacientes classificados como Ahlback I também apresentaram faixa etária maior do que aqueles com gravidade grau II, o que pode ser explicado pela maior demora no acesso ao tratamento médico devido ao estudo ter sido realizado em uma instituoção terciária.

A obesidade é um fator de risco para surgimento e desenvolvimento da osteoartrite no joelho. No nosso estudo, os pacientes avaliados apresentaram IMC em torno de 31,25 ± 5,71 e peso ao redor de 84,39 ± 16,69 kg, acima dos 76,5 kg encontrados nos estudos de Demirag et al[20]. Utilizando-se os critérios da OMS, observa-se que 34,5% dos indivíduos estavam na faixa de sobrepeso, e 55,4% da nossa população eram de pacientes com obesidade, superando os achados de outros estudos como de Kantor et al[12] (45% dos avaliados acima do peso e 17% com obesidade), de Reginato et al[17] (41,3% dos pacientes acima do peso e 38,2% com obesidade) e de Coimbra et al[21] (46% dos pacientes com obesidade e 30% com sobrepeso). Tais achados podem estar associados à menor mobilidade dos pacientes com OA, o que dificulta a redução ponderal pela prática de atividades físicas. Além disso, também pode ser justificado pela relação próxima entre a osteoartrite e a síndrome metabólica.

Apesar da maioria dos pacientes apresentar acometimento no joelho direito (55,6%), nosso trabalho mostrou não ter diferença significativa entre a lateralidade da articulação. Outros dois estudos, divergentes entre si, apresentam resultados contrários, já que encontraram maior acometimento no joelho esquerdo[22] e direito[23].

Quando estratificamos os pacientes pela gravidade da OA, verificamos que a maior parte dos pacientes incluídos se encontra nos graus II (49,6%) e III (28,9%) da classificação de Ahlback. Isso é justificado pelo perfil do programa de tratamento conservador da osteoartrite, indicado até o grau III de Ahlback. Em outro estudo, a OA radiográfica com graus 2 e 3 da escala KL foi encontrada em 39,4% e 40,8% dos indivíduos, respectivamente[17].

Encontramos maior proporção de pacientes com deformidade dos membros inferiores em varo naqueles com graus III (51,5%) e IV + V (66,7%) de OA. Tal achado já foi descrito em outras publicações, já tendo sido descrito que 82% dos pacientes com OA do joelho apresentam mau alinhamento dos membros inferiores, sendo a maioria em varo[24]. Da mesma forma, também foi encontrada maior proporção de pacientes com crepitação difusa nos pacientes com graus III (62,3%), IV e V (90,7%) de OA. Já foi descrito que pacientes com OA do joelho que apresentam crepitação possuem função levemente pior autorrelatada, menor qualidade de vida e maior dor em comparação àqueles sem crepitação[25]. A maior proporção de pacientes com presença de crepitação ao exame físico nos pacientes com maior gravidade de OA é compatível com o referido estudo, pois pacientes com maior gravidade, possuem pior função e qualidade de vida.

Ao avaliarmos a distribuição de pacientes tratados com condroprotetores orais ou viscossuplementação, observamos um predomínio por este último tratamento (56,7%). Tal fato pode ser justificado pelo fato de boa parte dos pacientes fazer uso de outras medicações, assim sendo optado pela viscossuplementação para evitar adicionar mais um medicamento àqueles já utilizados, reduzindo o risco de erros na utilização ou ainda interações medicamentosas. Além disso, o consenso brasileiro de viscossuplementação do joelho[26] considerou que a mesma pode ser utilizada como primeira linha de tratamento da OA do joelho, opinião que é corroborada pela equipe de ortopedistas da instituição.

Um estudo envolvendo internações hospitalares por osteoartrite evidenciou que o perfil da população mais afetada foi de mulheres da etnia branca, com faixa etária entre 60 e 69 anos, e da região sudeste[27]. Outro estudo, realizado entre idosos frequentadores de um centro de convivência demonstrou a presença de osteoartrite em um indivíduo a cada 12 estudados, com maior prevalência de nos frequentadores do sexo feminino, com idade acima de 55 anos e com sobrepeso[28]. Entretanto, ambos os estudos apresentavam tamanho amostral bem inferior ao deste estudo.

O ponto forte do presente estudo foi o fato de incluir pacientes de todos os graus de OA, em tratamento conservador. A grande maioria dos estudos foca nos pacientes submetidos à artroplastia do joelho de modo que somente inclui os pacientes com maior gravidade. Ao observarmos as características dos pacientes em graus iniciais, podemos pensar no estabelecimento de políticas públicas e pesquisas com foco nesse perfil de paciente, visando não apenas melhorar a qualidade de vida, mas mitigar as consequências da doença e até mesmo postergar procedimentos cirúrgicos. Além disso, temos como vantagem o fato

de os pacientes terem de sido acompanhados em um ambulatório especializado num mesmo centro, especializado em ortopedia, que recebe pacientes de todo o estado do Rio de Janeiro, representando assim uma parcela característica da população brasileira. Isso reflete a relevância do estudo, ainda que observacional, dada a escassez de dados epidemiológicos em brasileiros.

Como limitação do estudo identificamos que, apesar dos protocolos existentes na instituição, alguns prontuários apresentavam dados incompletos, limitando a coleta de dados, o que foi minimizado pelo tamanho do número de pacientes atendidos pelo programa. Por outro lado, o fato de a amostra incluir dados de pacientes de todo o estado, devido à abrangência nacional da instituição, consideramos que o estudo é representativo do perfil de acometimento da doença em todo o país, de modo que os resultados obtidos podem ser extrapolados para a população brasileira.

CONCLUSÃO

O perfil da população de pacientes com osteoartrite do joelho submetidos ao tratamento conservador em uma instituição terciária de saúde do SUS, em sua maioria é do sexo feminino, apresenta obesidade grau I e idade superior a 60 anos. Os graus II e III de Ahlback são preponderantes em relação à gravidade da osteoartrite.

REFERÊNCIAS

1. Huang PH, Wang CJ, Chou WY, Wang JW, Ko JY. Short-term clinical results of intra-articular PRP injections for early osteoarthritis of the knee. *Int J Surg*. 2017;42:117-22.
2. Bijlsma JWJ, Berenbaum F, Lafeber FPJG. Osteoarthritis: an update with relevance for clinical practice. *Lancet*. 2011;377(9783):2115-26.
3. Zheng K, Shen N, Chen H, Ni S, Zhang T, Hu M, et al. Global and targeted metabolomics of synovial fluid discovers special osteoarthritis metabolites. *J Orthop Res*. 2017;35(9):1973-81.
4. Bączkowicz D, Skiba G, Czerner M, Majorczyk E. Gait and functional status analysis before and after total knee arthroplasty. *Knee*. 2018;25(5):888-96.
5. Li X, Shah A, Franklin P, Merolli R, Bradley J, Busconi B. Arthroscopic debridement of the osteoarthritic knee combined with hyaluronic acid (Orthovisc) treatment: a case series and review of the literature. *J Orthop Surg Res*. 2008;3:43.
6. Guccione AA, Felson DT, Anderson JJ, Anthony JM, Zhang Y, Wilson PW, et al. The effects of specific medical conditions on the functional limitations of elders in the Framingham Study. *Am J Public Health*. 1994;84(3):351-8.
7. Bertolini FM, Leopoldino AAO, Mesquita JVD, Cousin E, Passos VMA. Increasing burden of osteoarthritis in Brazil from 2000 to 2017 – results from the Global Burden of Disease Study (GBD), 2017. *Acta Fisiatr*. 2020;27(2):76-81.
8. Associação Brasileira para o Estudo da Obesidade e Síndrome Metabólica. *Diretrizes brasileiras de obesidade*. São Paulo: ABESO; 2016.
9. Healey EL, Afolabi EK, Lewis M, Edwards JJ, Jordan KP, Finney A, et al. Uptake of the NICE osteoarthritis guidelines in primary care: a survey of older adults with joint pain. *BMC Musculoskelet Discord*. 2018;19(1):295.
10. Jordan JM, Helmick CG, Renner JB, Luta G, Dragomir AD, Woodard J, et al. Prevalence of knee symptoms and radiographic and symptomatic knee osteoarthritis in African Americans and Caucasians: the Johnston County Osteoarthritis Project. *J Rheumatol*. 2007;34(1):172-80.
11. Lawrence RC, Felson DT, Helmick CG, Arnold LM, Choi H, Deyo RA, et al. Estimates of the prevalence of arthritis and other rheumatic conditions in the United States. Part II. *Arthritis Rheum*. 2008;58(1):26-35.
12. Kantor ED, Lampe JW, Navarro S, Song X, Milne GL, White E. Associations between glucosamine and chondroitin supplement use and biomarkers of systemic inflammation. *J Altern Complement Med*. 2014;20(6):479-85.
13. Vårbakken K, Lorås H, Nilsson KG, Engdal M, Stensdotter AK. Relative difference among 27 functional measures in patients with knee osteoarthritis: an exploratory cross-sectional case-control study. *BMC Musculoskelet Disord*. 2019;20(1):462.
14. Lo GH, Ikpeama UE, Driban JB, Kriska AM, McAlindon T, Petersen NJ, et al. Evidence that swimming may be protective of knee osteoarthritis: Data from the Osteoarthritis Initiative. *PM R*. 2020,12(6):529-37.
15. Dorais M, Martel-Pelletier J, Raynauld JP, Delorme P, Pelletier JP. Impact of oral osteoarthritis therapy usage among other risk factors on knee replacement: a nested case-control study using the Osteoarthritis Initiative cohort. *Arthritis Res Ther.* 2018;20(1):172.
16. Lombnæs GØ, Magnusson K, Østerås N, Nordsletten L, Risberg MA, Hagen KB. Distribution of osteoarthritis in a Norwegian population-based cohort: associations to risk factor profiles and health-related quality of life. *Rheumatol Int*. 2017;37(9):1541-50.
17. Reginato AM, Riera H, Vera M, Torres AR, Espinosa R, Esquivel JA, et al. Osteoarthritis in Latin America: Study of Demographic and Clinical Characteristics in 3040 Patients. *J Clin Rheumatol*. 2015;21(8):391-7.
18. Instituto Brasileiro de Geografia e Estatística. *Censo demográfico 2010*. Rio de Janeiro: IBGE; 2010.
19. Gomes R, do Nascimento EF, de Araújo FC. Por que os homens buscam menos os serviços de saúde do que as mulheres? As explicações de homens com baixa escolaridade e homens com ensino superior. *Cadernos de Saúde Pública*. 2007;15(6):2859-69.
20. Demirağ MD, Özkan S, Haznedaroğlu S, Kilinç EA, Aksakal FNB, Aycan S, et al. Associations between obesity and the radiographic phenotype in knee osteoarthritis. *Turk J Med Sci*. 2017;47(2):424-9.

21. Coimbra IB, Plapter PG, de Campos GC. Generating evidence and understanding the treatment of osteoarthritis in Brazil: a study through Delphi methodology. *Clinics*. 2019;74:e722.

22. Komatsu D, Ikeuchi K, Kojima T, Takegami Y, Amano T, Tsuboi M, et al. Laterality of radiographic osteoarthritis of the knee. *Laterality*. 2017;22(3):340-53.

23. Neame R, Zhang W, Deighton C, Doherty M, Doherty S, Lanyon P, et al. Distribution of radiographic osteoarthritis between the right and left hands, hips, and knees. *Arthritis Rheum*. 2004;50(5):1487-94.

24. Felson DT. Osteoarthritis as a disease of mechanics. *Osteoarthritis Cartilage*. 2013;21:10-5.

25. Pazzinatto MF, Silva DO, Faria NC, Simic M, Ferreira PH, Azevedo FM, et al. What are the clinical implications of knee crepitus to individuals with knee osteoarthritis? AN observational study with data from the Osteoarthritis Initiative. *Braz J Phys Ther*. 2019;23(6):491-6.

26. de Campos GC, de Sousa EB, Hamdan PC, Almeida CS, Tieppo AM, Rezende MU, et al. Brazilian consensus statement on viscosupplementation of the knee (COBRAVI). *Acta Ortop Bras*. 2019;27(4):230-6.

27. de Souza FM, Matos MA, Rocha FA, Silveira Junior PFA, Stecca TM, Gehhlen SHJ, et al. Análise das características epidemiológicas e hospitalares da osteoartrite referente aos casos registrados no Brasil nos últimos 5 anos. *Res Soc Develop*. 2022;11(16):e292111638383.

28. Pancotte J, Bortoluzzi EC, Graeff DB, Sant'Anna Alves AL, Wibelinger LM, Doring M. Osteoartrite: prevalência e presença de fatores associados em idosos ativos. *Rev Cienc Med Biol*. 2017;16(1):40-4.

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Review Article

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CANDIDEMIA ASSOCIATED WITH COVID-19: RISK FACTORS AND PREDISPOSITION IN CRITICAL PATIENTS

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ABSTRACT

Introduction: Coronavirus diasease 19 (COVID-19) is an infection caused by the new coronavirus - SARS-CoV-2 in 2019. The infection quickly spread throughout the world, establishing itself like a pandemic. Reports on secondary fungal co-infections in critically ill patients COVID-19 are still scarce and their dynamics are poorly understood. Candidemia is defined as the presence of *Candida* species in one or more blood cultures, being one of the most reported opportunistic fungal infections in intensive care units (ICU).

Methods: Three databases were used for the literary search: Pubmed, Science Direct, and Scopus, including articles published between 2020 and 2021.

Results: The incidence of candidemia in COVID-19 patients ranged up to 12% of COVID-19 reported cases. *Candida albicans* was the most prevalent species, followed by non-*albicans* species. The use of broad-spectrum antimicrobials, corticosteroids, central venous catheters, mechanical ventilation, parenteral nutrition, immunosuppressants such as tocilizumab and prolonged hospital stay were predisposing factors for the candidemia in COVID-19 patients.

Conclusion: There are strongly established risk factors that influence the establishment of candidemia in critically ill COVID-19 patients, which contributes to increased mortality in these patients. Finally, active surveillance by the medical team should be maintained for previous signs of fungal co-infection associated with SARS-CoV-2 contamination.

Keywords: *COVID-19, candidemia, fungal co-infection, SARS-CoV-2, risk factors*

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INTRODUCTION

Coronavirus disease 19 (COVID-19) is an infection caused by a new coronavirus, SARS-CoV-2, which present rapid transmission and contagion between humans. The first reports of the infection appeared in Wuhan, China, in late 2019. Consequently, COVID-19 has quickly been declared a pandemic by the World Health Organization (WHO)[1]. Currently, Brazil ranks third in the world in the total number of cases of infection and the second in several deaths. Reports on secondary infections in patients with an initial diagnosis of contamination by SARS-CoV-2 are still very limited[2,3]. The establishment of a secondary infection associated with COVID-19 can aggravate the inflammatory process in these patients, contributing to the increase of the pathogenicity and influencing a poor prognosis.[4]

Candidemia, defined as the presence of *Candida* spp. in one or more blood cultures, is one of the most common opportunistic fungal infections in intensive care units (ICU). Despite the diversity of species that the genus presents, cases of infection are primarily attributed to five species: *C. albicans*, *C. glabrata*, *C. tropicalis*, *C. parapsilosis*, and *C. krusei*[5,6]. External infections in medical devices, such as catheters and probes, can be the primary source of cases of candidemia. However, endogenous sources can also cause infection[7-9].

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In most cases of candidemia, antifungal therapy is not responsive. Thus, infection is associated with increased length of stay, a high cost to the health system and high mortality, between 40-60% of cases[10-12]. The most common risk factors for the establishment of candidemia include critical illness and prolonged ICU stay. Other risk factors, such as the presence of medical devices, exposure to corticoids and antibiotics, surgery, malignant tumors, acute necrotizing pancreatitis, organ transplants, and parenteral nutrition, can also contribute to the infection development[13,14].

From the exposure scenario, COVID-19 patients admitted to intensive care units (ICU) are more likely to the establishment of secondary fungal infections, such as candidemia. Thus, it is important to identify and address modifiable risk factors in an attempt to prevent and reduce the occurrence of candidemia in COVID-19 patients. Thus, this study evaluated to address risk factors in critically ill COVID-19 patients in establishing *Candida* spp. causing a secondary infection, in addition to providing a basis for a better understanding of this problem.

METHODOLOGY

For this study, the articles published in the area of interest were identified in three different databases: Pubmed, Science Direct, and Scopus. As inclusion criteria, english articles published between 2020 and 2021 and using the following keywords, alone or in combination: candidemia, covid 19, invasive candidiasis, coronavirus were selected. The studies were critically reviewed, excluding those that do not correspond to the theme delimitation and duplicate articles. In a total, 17 studies were selected and analyzed (Figure 1).

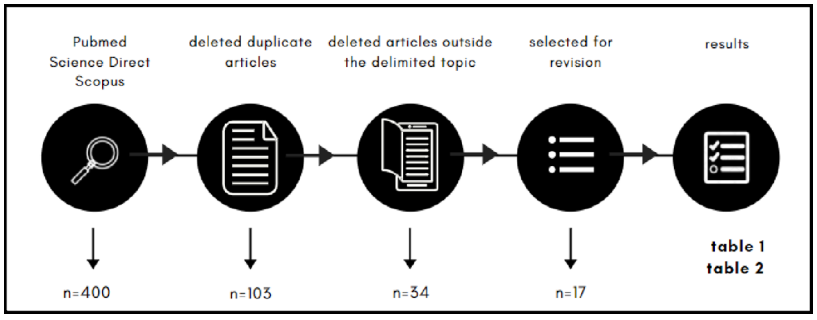

Figure 1: Scheme sequence of the methodology used to select the analyzed articles.

IMMUNE RESPONSE EXPRESSION ASSOCIATED WITH CANDIDEMIA IN COVID-19 PATIENTS

Candida spp. is present in a commensal manner of the mucosal microbiota of approximately 50-70% of immunocompetent individuals under normal conditions. Changes in the individual's immune response due to external factors or the imbalance of intestinal barriers, such as in surgeries involving the gastrointestinal tract (GIT), can spread through the abdominal cavity, developing an opportunistic infection, such as candidemia[9,11].

Neutrophils play an important role in the innate immune response to fungal pathogens, as they are the first cells to be recruited through pro-inflammatory cytokines[9,15,16]. Thus, neutropenia is an established risk factor for both dissemination candidemia and patient mortality[11]. Monocytes and macrophages also perform another principal function, as they can phagocytose and eliminate yeast, secreting cytokines and inflammatory factors that contribute to the host's immune respons. A study by Ngo[17] showed that mice who are deficient in phagocytic cells had more proliferation and lethality by *C. albicans* than the control group[17,18] NK cells also represent a major component of the innate immune system. Studies demonstrate the ability of these cells to control fungal infection[19,20], despite that their participation in the individual's immune response against fungi is still poorly understood.

SARS-CoV-2 infection promotes an innate immune response that induces an excessive inflammatory process, known as "cytokine storm". The *Candida* genus has specific virulence factors that allow the establishment of opportunistic infection when the individual is in a picture of immunosuppression, observed in patients with associated risk factors. The disruption of the enterocyte epithelial barrier, caused by SARS-CoV-2, can facilitate the translocation of *Candida* species from the intestinal lumen to the bloodstream alteration in the host's defense mechanisms caused by the virus allows the colonization and installation of secondary infection by *Candida* spp[21,22]. Although the immune system is compromised in COVID-19 patients, the immune cells, responsible for the innate immune response to *Candida* spp., are still present. Thus, it is possible that the risk factors related to the patient's clinic are crucial and determine substances for the development and installation of candidemia cases[23].

Tocilizumab is a recombinant monoclonal antibody that binds to the membrane of interleukin-6 [IL-6], preventing binding to the enzyme receptor and inhibiting the activation of the inflammatory cascade[24]. The use of tocilizumab is based on the hypothesis that it supposedly decreases the rate of mortality caused by SARS-CoV-2 is still being studied, as there are conflicting results among several clinical trials reported[24-26]. In this sense, the use of this drug may be associated with an increase in invasive fungal infections, as it is an immunosuppressant and its adverse effect is neutropenia, a known risk factor for candidemia[27].

Taramasso et al. evaluated the administration of tocilizumab in critically ill COVID-19 patients[28]. The group of patients who received the drug and developed secondary infections showed no significant changes in laboratory parameters, only reduced C-Reactive Protein (CRP) parameters when compared to patients who did not receive the drug, which is commonly observed when using treatment with immunosuppressants. Because of this, when there is suspicion of secondary infections in patients who used tocilizumab, the medical approach should not be based only on examinations of inflammatory signs, as these may not be completely reliable for the patient's condition. The study also mentions that the drug did not change manifestation of infection symptoms, such as fever, hypothermia, and hypotension. According to y Al-Baadani et al., among the group of patients who used tocilizumab (n=62), 10 patients developed secondary infections: 5/10 hospital pneumonia associated with mechanical ventilation and another five patients, bloodstream infections, with candidemia being reported in 4/5 of the cases[26] (Table 1).

According to Antinori et al., the use of tocilizumab was considered a risk factor for the development of candidemia[25]. All patients received a dose of 8mg/kg of the drug and had a mean time of 13 days between the last administration and the development of infection (Table 1). Seeing the immune response may be altered after using the drug, the presence of an inflammatory response associated with symptoms of fever and high serum levels of CRP, which are characteristic of clinical suspicion of infections, may be minimized. Therefore, medical professionals must be on active surveillance for the risk of candidemia in these patients.

NOTIFICATION OF CANDIDEMIA CASES IN COVID-19 PATIENTS

COVID-19-associated candidiasis (CAC) has been registered and reported in several countries, with different incidences between different continents, ranging from 0.7 - 12%. The difference between the pandemic management protocols between each country, and the difficulty in diagnosing secondary infections, means that the manifestation of fungal infections remains under-reported[29].

In this study, countries from all continents were not included, which can explain due to some factors: inadequate studies or insufficient data for analysis, different managements during the pandemic between governments, contact isolation protocol making it difficult to diagnose underlying infections for later notifications and the urgency to face the seriousness of the social and economic effects caused by the health crisis. Thus, what is available from the report and published studies are a sample of cases. However, some factors make it impossible to have a panoramic and global view of the problem associated with the COVID-19 pandemic (Figure 2).

Figure 2: Geolocation of candidemia case studies in COVID-19 patients presented in Table 1 (United States, Greece, India, Oman, Brazil, Italy, United Kingdom, Spain, Turkey, Iran).

CANDIDEMIA EPIDEMIOLOGY IN COVID-19 PATIENTS

Among the fungal infections acquired in hospital environments, the *Candida* genus is the most prevalent and is among the major pathogens involved in bloodstream infections in ICU'S[30,31]. Worldwide, *C. albicans* is the most prevalent species in blood cultures, accounting for approximately 40 to 60% of cases, although the rates of candidemia caused by *Candida* non-albicans have increased due to the high mortality rates in standard patients[32,33]. Following this pattern, in most of the countries included, *C. albicans* was the most prevalent species in COVID-19 patients who developed candidemia, followed by non-albicans species, varying by continental region. Of these, *C. krusei* and *C. dubliniensis* were mentioned less prevalently (Table 1).

C. albicans is the most prevalent species United States of America (USA) ICU, followed by *C. glabrata* and *C. parapsilosis*[34]. This is in agreement with Macauley et al.[35] and Seagle et al[36]. studies, that related to the incidence of patients who developed CAC[35,36]. In Spain, *C. albicans* was the most prevalent species in COVID-19 patients with candidemia, followed by *C. parapsilosis* and *C. glabrata* (Table 1) and are in accord with the epidemiology of the genus in patients with candidemia in the country[5]. The difference in the epidemiological pattern of species between continents may be related to the variation of important factors such as climate, antifungal treatment, and protocols related to the use of central venous catheters in patients.

In Latin America, *C. albicans* also remained the most prevalent species, *C. tropicalis* and *C. glabrata* ranged between the second and third most common isolate. The emerging incidence of non-albicans in recent years poses a problem since they have low susceptibility to fluconazole[37]. In Brazil, in a group of COVID-19 patients who developed candidemia, *C. glabrata* was the most prevalent species among non-*albicans Candida,* followed by *C. tropicalis*. *C. auris* was reported by Chowdhary et al.[38] and Magnasco et al.[39] and related to nosocomial infection that culminated in local outbreaks of candidemia in COVID-19 patients[38,39]. This may be related to the ease of transmission in hospital environments. The standard of care and the need for intensive care in severe cases of COVID-19 make these patients more susceptible to the colonization of this yeast. This situation is alarming, as it has high rates of resistance to available clinical treatment, such as fluconazole and other antifungals[38].

COVID-19 THERAPY AS A RISK FACTOR FOR THE CANDIDEMIA ESTABLISHMENT

The corticosteroids use is described as a risk factor for the fungal infection establishment of fungal, as it can promote exacerbated fungal growth, is associated with an increased risk of establishing systemic infections, such as candidemia[40]. Corticosteroids are used to treat a variety of symptoms and diseases, including in the COVID-19 treatment. Dexamethasone, for example, is used in combination therapy with antimicrobials because it exerts an important inhibitory effect on inflammatory factors, being about 25 times more active than other drugs in the same group. The main effect of dexamethasone is the inhibition of a pro-inflammatory gene that encodes chemokines, cytokines, and adhesion molecules to the acute inflammatory response, mainly affecting two aspects: chemotaxis and vasodilation[41-43].

Studies associating corticosteroids with antifungal therapy have shown changes in the patient's response after the introduction of corticosteroids, with a deleterious effect on treatment[44-46]. Corticosteroids are a therapeutic alternative for pneumonia caused by SARS-CoV-2, but it is important to determine protocols for treatment and the right time to start these interventions. High doses of corticosteroids may be related to the increased frequency of candidemia in COVID-19 patients. Medical professionals should be aware of the potential for establishing systemic infections in critically ill patients[40].

The use of broad-spectrum antimicrobials in hospitalized patients, both in targeted treatments and in empirical treatment, can alter the normal microbiota, promoting the overgrowth of opportunistic fungi[6]. Since the beginning of the pandemic, several studies have shown an increase in prescriptions of antimicrobials. During the first global wave of COVID-19, the use of broad-spectrum antibiotics was observed in more than 70% of cases, even with literature data showing that bacterial co-infection occurs in less than 10% of cases[47]. The indiscriminate use of antimicrobials in the absence of a well-established protocol may be contributing to the increase in cases of candidemia observed in COVID-19 patients[48]. The selection of bacterial and fungal isolates resistant to antimicrobial therapy has become a problem worldwide, justifying the emergence of programs for the rational use of medicines and the establishment of infection control measures[39].

Seeing that some intestinal bacteria regulate the growth of pathogenic microorganisms and also the proliferation of yeasts, the indiscriminate and undirected use of broad-spectrum antimicrobials favors the opportunistic growth of *Candida* spp. As can be seen in the studies described (Table 1), the use of broad-spectrum antibiotics and the use of corticosteroids in the therapeutic protocol of COVID-19 patients is extremely prevalent and related to an important risk factor in the development of candidemia.

RISK FACTORS FOR THE ESTABLISHMENT OF CANDIDEMIA IN CRITICAL ICU PATIENTS

Approximately 5 to 30% of patients diagnosed positive for SARS-CoV-2 require admission to the ICU. Widely known risk factors for the development of candidemia are present in these patients, such as mechanical ventilation, parenteral nutrition, and the use of a central venous catheter[49]. In our study, these widely known risk factors for invasive *Candida* infection were cited in the absolute majority of the studies analyzed.

Catheter-related disseminated infections can originate from various pathways, such as through the patient's skin microbiota, exogenous contamination in the handling of healthcare professionals, or contamination from infusions[50]. *Candida* spp. it is the third most prevalent etiologic agent in central venous catheter-related infections[51]. These devices are highly associated with the development of candidemia, as the *Candida* genus is a colonizer of this type of access, as it provides an adhesion surface for yeast colonization in these sites forming biofilms[31,52].

An epidemiological study by Fram carried out by a university hospital in São Paulo during the pandemic revealed that candidemia was the most prevalent bloodstream infection associated with central venous catheters in ICU's in 2020, with approximately 29.1% of the reported infections cases[53]. *Candida* spp. it is among the most commonly isolated pathogens from the biofilm formed in the endotracheal tube and tracheal secretions in patients with ventilator-associated pneumonia[54]. Central venous catheters should be removed in patients with candidemia if this situation is clinically feasible. When removal is not possible, therapy with echinocandin or amphotericin B should preferably be performed, as they are effective against biofilms[55].

Parenteral nutrition (PN) is commonly identified as a risk factor for the development of candidemia. PN causes important changes in the patient, such as the alteration of the normal intestinal microbiota from gram-positive bacteria to a predominance of gram-negative bacteria, alters the barrier function of epithelial cells, and causes inflammation. These factors turn the intestine into a favorable environment for colonization by *Candida* spp.[12] Another hypothesis is related to the development of hyperglycemia due to the high concentration of dextrose caused by continuous infusion, which can impair and reduce the immune response of critical patients, leading to secondary infections[50].

The prolonged length of stay in the ICU's is also considered a risk factor for the establishment of opportunistic infections. According to Bishburg et al.[34], COVID-19 patients without candidemia were hospitalized for an average of 10 days[34]. On the other hand, in the group of patients with CAC, the average hospital stay was 40 days. This behavior was also observed by Macauley et al.[35], since patients in the CAC group remained hospitalized significantly longer until the development of candidemia on average 19 days[35].

Table 1: Outcome of studies approach cases of candidemia in COVID-19 patients published between 2020 - 2021.

Authors/Country	Isolated	Main results	Risk factors
Arastehfar, 2021[56] (Iran)	*C. albicans* *C. glabrata*	In the period analyzed (November/2020 to January/2021), 1988 patients were diagnosed with COVID-19 and admitted to the hospitals. Seven patients developed fungemia (0.03%), of which 6/7 had candidemia. All patients with CAC died despite treatment with antifungal agents. This led to an extremely high mortality rate in COVID-19 patients when compared to patients without candidemia (452/1988).	Central venous catheter Broad spectrum antibiotics ICUs Parenteral nutrition Mechanical ventilation
Macauley & Epelbaum, 2021[35] (United States)	*C. albicans* *C. glabrata* *C. parapsilosis* *C. tropicalis* *C. krusei*	Fifty patients with candidemia were identified, with an incidence rate of approximately 13/1000 admissions. In the non-COVID group, 38 patients with candidemia and 12 in the COVID group were identified. The corresponding incidence (CI) rates were 11/1000 admissions in non-COVID and 51/1000 admissions in the COVID group. The mortality rate in the non-COVID group was 61%, while in the COVID group this rate reached 75% of cases.	Mechanical ventilation Central venous Catheter broad spectrum Corticosteroid therapy Days in ICU Hemodialysis

Continues...

Table 1: Continuation.

Authors/Country	Isolated	Main results	Risk factors
Kayaaslan, 2021[57] (Turkey)	*C. albicans* *C. glabrata* *C. parapsilosis* *C. tropicalis*	The incidence of candidemia in the COVID-19 group was 2.16%. The time for the development of fungal infection was shorter (approximately two weeks), the mortality rate was higher (92.5%) when compared to the non-COVID group, which had a CI of 1.06% and a mortality rate of 79.4 %.	Broad spectrum antibiotics Corticosteroid therapy CCentral venous catheter Mechanical ventilation Hemodialysis Surgery
Riche, 2020[40] (Brazil)	*C. albicans* *C. glabrata* *C. tropicalis*	During the study period, the incidence of candidemia was 1.43 (hospital 1) and 1.15 (hospital 2) in non-COVID-19 patients per 1000 patients/day. When compared to the previous year (pre-pandemic), these incidences remained unchanged. On the other hand, in the COVID-19 group of patients, the incidence was 11.83 (hospital 1) and 10.23 (hospital 2) per 1000 patients/day and during the same period analyzed. The mortality rate of patients with candidemia in the covid-19 group was 72.7% (8/11).	Corticosteroids Central venous catheter Broad spectrum antibiotics
Nucci. 2021[22] (Brazil)	*C. albicans* *C. glabrata* *C. tropicalis*	During the study period, 41 episodes of candidemia were observed Hospital days: 16 in period 1 (pre-pandemic) and 25 in period 2 Mechanical ventilation (during the pandemic). Of this total, nine cases in COVID-19 Central venous catheter patients. In period 2, the incidence of candidemia per 1000 admissions was 4.76 in patients without COVID-19 and 2.68 when considering cases of candidemia occurring in COVID-19 patients. The hospital average at an incidence of 1.3 per 1000 admissions over the years.	Hospital days Central venous catheter Mechanical ventilation
Abdullah, 2020[58] (Oman)	*C. albicans,* *C. glabrata,* *C. tropicalis*	Five cases of candidemia were reported in the adult ICU. All five patients had CVC and received broad-spectrum antibiotics. Persistent candidemia occurred in one patient even after removal of the CVC and administration of antifungal therapy. Despite appropriate management, 3/5 patients died.	Hospital days Central venous catheter Surgery Broad spectrum antibiotics Mechanical ventilation
Mastrangelo, 2020[59] (Italy)	*C. albicans*	In 21 patients belonging to the COVID group and 51 patients to the control group, the incidence rate of candidemia per 10,000 cases was significantly higher in the first group (10.97 versus 1.48.)	Days in ICU Immunosuppressants
Giacobbe, 2020[60] (Italy)	*C. albicans* *C. glabrata* *C. parapsilosis*	Seventy-eight critically-ill patients were included in the study 45 episodes of bloodstream infections were observed in 31 patients, with an incidence rate of 47 episodes per 1000 patient-days. Of these, 3/45 episodes were candidemia, representing 6.67% of cases.	-----

Continues...

Table 1: Continuation.

Authors/Country	Isolated	Main results	Risk factors
Antinori, 2020[25] (Italy)	*C. albicans* *C. tropicalis* *C. parapsilosis*	During 11 days, 43 COVID-19 patients received drug therapy with tocilizumab in the ICU and wards. Of these, three patients (6.9%) developed candidemia. Patients received an 8 mg/kg dose of tocilizumab (maximum 800 mg/d) repeated within 12 h after the first administration. The median time since the last dose of tocilizumab and the diagnosis of candidemia was 13 days.	Tocilizumab Parenteral nutrition Broad spectrum antibiotics
White, 2020[61] (United Kingdom)	*C.albicans, C.parapsilosis*	A total of 135 adults were analyzed and included in the study. 12.6% (17 patients) had fungal yeast infections. Of this group, 14 cases were invasive *Candida* spp. All patients were on mechanical ventilation. Of these, 6/15 died within 30 days of diagnosis.	Mechanical ventilation Central venous catheter
Bishburg, 2020[34] (United States)	*C. albicans, C. tropicalis* *C. parapsilosis* *C. glabrata*	During the study period, 89 patients were included in the COVID-19 group. The incidence of candidemia was 8/89 (8.9%) and a mean ICU stay of 25 days. Compared to patients without candidemia, patients with candidemia remained in the ICU longer (10 vs. 40 days).	Central venous catheter Broad spectrum antibiotics Corticosteroids Tocilizumab Mechanical ventilation
Agrifoglio, 2020[62] (Spain)	*C. albicans, C. parapsilosis* *C. glabrata*	Among 139 patients admitted and analyzed in this study, 15 cases of candidemia were reported (10.8% of cases). This group had a mortality rate of 40%.	Mechanical ventilation Central venous catheter Vasoactive drugs Parenteral nutrition Use of broad spectrum antibiotics Corticosteroids
Kokkoris, 2021[3] (Greece)	*C. albicans, C. parapsilosis*	Among 50 patients included during the study period, 7/50 (14%) developed candidemia. Candidemia was more frequent in patients who had bacteremia.	Mechanical ventilation Use of broad spectrum antibiotics
Denny, 2021 (United Kingdom)	*C. albicans* *C. parapsilosis* *C. glabrata* *C.dubliniensis*	In the period analyzed, 11 cases of candidemia were identified. An average of 14.8 days was observed for the development of secondary infection after the diagnosis of COVID-19. Thus, the 30-day mortality rate was 54.4% (6/11).	Mechanical ventilation Use of broad spectrum antibiotics Hemofiltration Central venous catheter
Magnasco, 2021[39] (Italy)	*C. auris*	During the period analyzed by the study, 118 patients were admitted to the COVID-19 ICU. Of this group, in 6/118 - approximately 5.1% of the cases, the etiological agent was *C. auris*. Out of 4/6 patients developed candidemia. The average time from admission to the first case of candidemia was 38 days, with a mortality rate of 50% (n=2).	Use of broad spectrum antibiotics
Chowdhary, 2020[38] (India)	*C. auris* *C. albicans* *C. tropicalis* *C. krusei*	A total of 596 COVID-19 patients were admitted. Candidemia was detected in 15/596 patients (2.5% of cases). Among these, 8/15 (53%) died. The mortality rate for C. auris was 60%.	Central venous catheter Mechanical ventilation

Continues...

Table 1: Continuation.

Authors/Country	Isolated	Main results	Risk factors
Seagle, 2021[36] (United States)	*C.albicans* *C. glabrata* *C.parapsilosis* *C. tropicalis* *C. lusitaniae* *C. dubliniensis*	Of 251 cases of candidemia included in the study, 64/251 were COVID-19 patients, with a median time of 15 days between positive diagnosis for COVID-19 and positive culture for *Candida* spp.	Central venous catheter Mechanical ventilation

ANTIFUNGAL THERAPY FOR CANDIDEMIA AND COVID-19 PATIENTS

The basis of antifungal treatment for candidemias is to use the class of azoles. However, the use often empirically has led to increased selection of resistant isolates, especially among non-albicans *Candida*. Fluconazole is the drug of choice and is widely used for the treatment of infections caused by *Candida* spp. However, *C. krusei* and *C. glabrata* are intrinsically resistant and, in this case, the echinocandins (anidulafungin, caspofungin, and micafungin) or amphotericin B are used. In particular, the treatment for *C. auris* must be done carefully, as there are reports of lack of susceptibility to azoles and amphotericin B, which hinders and limits available therapeutic options[52,56].

In a randomized study, anidulafungin was shown to be equivalent to fluconazole; later, in a second analysis, it showed a higher response rate to the treatment (fluconazole about 60%, anidulafungin 76%). Fluconazole should be administered at a high initial dose (800 mg/d), and may also be used for continuation of oral treatment after initial treatment with echinocandins. In most cases, voriconazole does not offer advantages when compared to fluconazole - except for *C. krusei* infections or in cases of suspected filamentous fungus infection[11,55,57,58]. Voriconazole is a second-generation triazole that has high efficacy in the treatment of disseminated fungal infections, with less toxicity compared to other antifungals such as amphotericin B (Figure 3)[59].

Despite treatment with antifungal agents, the mortality rate associated with candidemia is high. Echinocandin-resistant *C. glabrata* isolates and multidrug-resistant *C. auris* isolates can lead to therapeutic failure, in addition to the risk of causing outbreaks in critically ill patients, which makes the challenge in the appropriate therapeutic approach for these patients even greater[60,61]. The development of specific protocols for epidemiological surveillance, such as periodic screening for colonization for *Candida* spp. in hospital spaces; the assessment of risk factors associated with each patient; the introduction of protocols that enable early diagnosis; and limiting the initiation of empirical fungal treatment according to the patient's condition and local epidemiology may contribute to the reduction in the selection of fungal resistance[62].

According to the Brazilian guideline for the candidemia treatment, non-neutropenic patients can use echinocandins (caspofungin 70 mg initial dose, followed by 50 mg/day), micafungin 100 mg or anidulafungin 200 mg initial dose followed by 100 mg/day) and they are strongly recommended; fluconazole 800 mg (12 mg/kg) followed by 400 mg is also strongly recommended and amphotericin B (3–5 mg/kg/d) is strongly recommended[62]. For neutropenic patients, the same dose and treatment of non-neutropenic patients can be used, only fluconazole 800 mg (12 mg/kg), followed by 400 mg, is not recommended. Daily blood culture collections are recommended, candidemia should be treated for at least two weeks after negative blood cultures. Duration of treatment depends on patient follow-up and response to antifungal therapy[55]. In candidemia associated with COVID-19, fluconazole is the first therapeutic choice, followed by echinocandins and amphotericin B (Table 2). The therapeutic management chosen is adequate to proposee by literature for these cases (Figure 3).

Critically ill COVID-19 patients have the concomitant presence of predisposing factors to the development of opportunistic co-infections, such as the use of broad-spectrum antimicrobials, corticosteroids, central venous catheters, mechanical ventilation, parenteral nutrition, use of immunosuppressants such as tocilizumab and time of prolonged hospital stay. The incidence and reporting of candidemia cases in COVID-19 patients varied up to 12% of the cases. Overall, the candidemia epidemiology in patients who developed CAC maintained *C. albicans* as the most prevalent species, followed by *C. glabrata*, *C. parapsilosis*, and *C. tropicalis*, varying according to the country. Infections by *C. auris* in critically ill COVID-19 patients were also observed, which is a worrying factor, as it has multi-resistance against currently available antifungal therapies. Fluconazole was used as the first choice in the CAC treatment, followed by echinocandins and amphotericin B. The development of candidemia in critically ill COVID-19 patients leads to increased mortality associated with the infection, even in patients who received antifungal treatment. Thus, we highlight the importance of the medical team in the active surveillance of hospital units, as these patients are at greater risk for the development of opportunistic fungal co-infections.

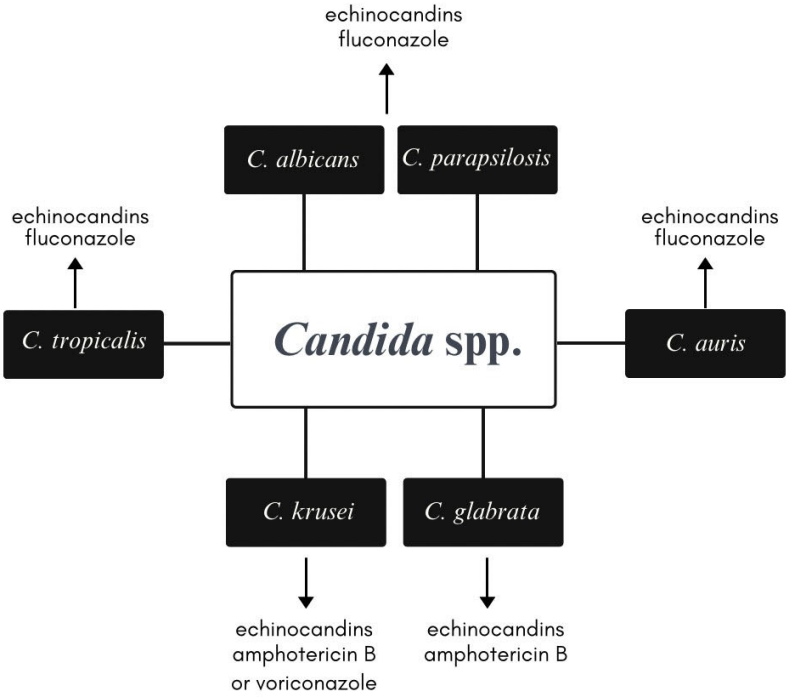

Figure 3: Suggestion treatment according to the species in the therapeutic management proposed by the Infectious Diseases Society of America from the isolation of *Candida* spp.

Table 2: Therapy administered in cases of candidemia.

Author/year	Treatment
Arastehfar (2021)	fluconazole, caspofungin
Riche (2020)	anidulafungin, fluconazole, amphotericin B, voriconazole.
Nucci (2021)	anidulafungin and fluconazole.
Abdullah (2020)	caspofungin, amphotericin B, voriconazole.
Antinori (2020)	caspofungin, fluconazole.
White (2020)	fluconazole, caspofungin, amphotericin B.
Bishburg (2020)	fluconazole, caspofungin.
Denny (2021)	fluconazole.
Magnasco (2021)	caspofungin, amphotericin B

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Conflict of interest

All authors declare to have no conflict of interest.

REFERENCES

1. Hoque MN, Chaudhury A, Akanda MAM, Hossain MA, Islam MT. Genomic diversity and evolution, diagnosis, prevention, and therapeutics of the pandemic COVID-19 disease. *PeerJ*. 2020;8:e9689. https://doi.org/10.7717/peerj.9689

2. Soriano MC, Vaquero C, Ortiz-Fernández A, Caballero A, Blandino-Ortiz A, Pablo R. Low incidence of co-infection, but high incidence of ICU-acquired infections in critically ill patients with COVID-19. *J Infect*. 2020;85(2):e20-e21. https://doi.org/10.1016/j.jinf.2020.09.010

3. Kokkoris S, Papachatzakis I, Gavrielatou E, Ntaidou T, Ischaki E, Malachias S, et al. ICU-acquired bloodstream infections in critically ill patients with COVID-19. *J Hosp Infect*. 2021;107:95-97. https://doi.org/10.1016/j.jhin.2020.11.009

4. Chen X, Liao B, Cheng L, Peng X, Xu X, Li Y, et al. The microbial coinfection in COVID-19. *Appl Microbiol Biotechnol*. 2020;104(18):7777-85. https://doi.org/10.1007/s00253-020-10814-6

5. Guinea J. Global trends in the distribution of Candida species causing candidemia. *Clin Microbiol Infect*. 2014;20(Suppl 6):5-10. https://doi.org/10.1111/1469-0691.12539

6. Görkem A, Sav H, Kaan Ö, Eren E. Coronavirus disease and candidemia infection: a case report. *J Mycol Med*. 2021;31(3):101155. https://doi.org/10.1016/j.mycmed.2021.101155

7. Eggimann P, Garbino J, Pittet D. Epidemiology of Candida species infections in critically ill non-immunosuppressed patients. *Lancet Infect Dis*. 2003;3(11): 685-702.

8. Pfaller MA, Diekema DJ. Epidemiology of invasive candidiasis: a persistent public health problem. *Clin Microbiol Rev*. 2007;20(1):133-163.

9. Teoh F, Pavelka N. How chemotherapy increases the risk of systemic candidiasis in cancer patients: current paradigm and future directions. *Pathogens*. 2016;5(1):6. https://doi.org/10.3390/pathogens5010006

10. Antinori S, Milazzo L, Sollima S, Galli M, Corbellino M. Candidemia and invasive candidiasis in adults: a narrative review. *Eur J Intern Med*. 2016;34:21-8. https://doi.org/10.1016/j.ejim.2016.06.029

11. Pappas PG, Lionakis MS, Arendrup MC, Ostrosky-Zeichner L, Kullberg BJ. Invasive candidiasis. *Nat Rev Dis Primers*. 2018;4:18026.

12. Poissy J, Damonti L, Bignon A, Khanna N, Kietzell MV, Boggian K, et al. Risk factors for candidemia: a prospective matched case-control study. *Crit Care*. 2020;24(1):109. https://doi.org/10.1186/s13054-020-2766-1

13. Pongrácz J, Juhász E, Iván M, Kristóf K. Significance of yeasts in bloodstream infection: epidemiology and predisposing factors of Candidaemia in adult patients at a university hospital (2010-2014). *Acta Microbiol Immunol Hung*. 2015;62(3):317-29.

14. Tadec L, Talarmin JP, Gastinne T, Bretonnière C, Miegeville M, Pape P, et al. Epidemiology, risk factor, species distribution, antifungal resistance and outcome of Candidemia at a single French hospital: a 7-year study. *Mycoses*. 2016;59(5):296-303.

15. Rudkin FM, Bain JM, Walls C, Lewis LE, Gow NAR, Erwig LP. Altered dynamics of Candida albicans phagocytosis by macrophages and PMNs when both phagocyte subsets are present. *mBio*. 2013;4(6):e0081013.

16. Netea MG, Joosten LAB, van der Meer JWM, Kullberg BJ, van de Veerdonk FL. Immune defence against Candida fungal infections. *Nat Rev Immunol*. 2015;15(10):630-42.

17. Ngo LY, Kasahara S, Kumasaka DK, Knoblaugh SE, Jhingran A, Hohl TM. Inflammatory monocytes mediate early and organ-specific innate defense during systemic candidiasis. *J Infect Dis*. 2014;209(1):109-19.

18. Zhou Y, Cheng L, Lei YL, Ren B, Zhou X. The interactions between Candida albicans and mucosal immunity. *Front Microbiol*. 2021;12:652725. https://doi.org/10.3389/fmicb.2021.652725 .

19. Voigt J, Hünniger K, Bouzani M, Jacobsen ID, Barz D, Hube B, et al. Human natural killer cells acting as phagocytes against Candida albicans and mounting an inflammatory response that modulates neutrophil antifungal activity. *J Infect Dis*. 2014;209(4):616-26.

20. Quintin J, Voigt J, van der Voort R, Jacobsen ID, Verschueren I, Hube B, et al. Differential role of NK cells against Candida albicans infection in immunocompetent or immunocompromised mice. *Eur J Immunol*. 2014;44(8):2405-14.

21. Arastehfar A, Carvalho A, Nguyen MH, Hedayati MT, Netea MG, Perlin DS, et al. COVID-19-Associated Candidiasis (CAC): an underestimated complication in the absence of immunological predispositions? *J Fungi*. 2020;6(4):211. https://doi.org/10.3390/jof6040211

22. Nucci M, Barreiros G, Guimarães LF, Deriquehem VAS, Castiñeiras AC, Nouér SA. Increased incidence of candidemia in a tertiary care hospital with the COVID-19 pandemic. *Mycoses*. 2021;64(2):152-156. https://doi.org/10.1111/myc.13225.

23. Roudbary M, Kumar S, Kumar A, Černáková L, Nikoomanesh F, Rodrigues CF. Overview on the prevalence of fungal infections, immune response, and microbiome role in COVID-19 patients. *J Fungi*. 2021;7(9):720. https://doi.org/10.3390/jof7090720

24. Zhang C, Wu Z, Li JW, Zhao H, Wang GQ. Cytokine release syndrome in severe COVID-19: interleukin-6 receptor antagonist tocilizumab may be the key to reduce mortality. *Int J Antimicrob Agents*. 2020;55(5):105954.

25. Antinori S, Bonazzetti C, Gubertini G, Capetti A, Pagani C, Morena V, et al. Tocilizumab for cytokine storm syndrome in COVID-19 pneumonia: an increased risk for candidemia? *Autoimmun Rev*. 2020;19(7):102564.

26. Al-Baadani A, Eltayeb N, Alsufyani E, Albahrani S, Basheri S, Albayat H, et al. Efficacy of tocilizumab in patients with severe COVID-19: survival and clinical outcomes. *J Infect Public Health*. 2021;14(8):1021-27.

27. Mo Y, Adarkwah O, Zeibeq J, Pinelis E, Orsini J, Gasperino J. Treatment with tocilizumab for patients with COVID-19 infections: a case-series study. *J Clin Pharmacol*. 61(3):406-11. https://doi.org/10.1002/jcph.1787

28. Taramasso L, Magnasco L, Portunato F, Briano F, Vena A, Giacobbe DR, et al.. Clinical presentation of secondary infectious complications in COVID-19 patients in intensive care unit treated with tocilizumab or standard of care. *Eur J Intern Med*. 2021;94:39-44.. https://doi.org/10.1016/j.ejim.2021.08.020

29. Kordalewska M, Guerrero KD, Garcia-Rubio R, Jiménez-Ortigosa C, Mediavilla JR, Cunningham MH, et al.. Antifungal drug susceptibility and genetic characterization of fungi recovered from COVID-19 patients. *J Fungi*. 2021;7(7):552.

30. Segrelles-Calvo G, Araújo GRS, Frases S. Systemic mycoses: a potential alert for complications in COVID-19 patients. *Future Microbiol*. 2020;15(14):1405-13. https://doi.org/10.2217/fmb-2020-0156

31. Zuo XS, Liu Y, Cai X, Zhan L, Hu K. Association of different Candida species with catheter-related candidemia, and the potential antifungal treatments against their adhesion properties and biofilm-forming capabilities. *J Clin Lab Anal*. 2021;35 (4):e23738. https://doi.org/10.1002/jcla.23738.

32. Epelbaum O, Chasan R. Candidemia in the Intensive Care Unit. *Clin Chest Med*. 2017;38(3):493-509. https://doi.org/10.1016/j.ccm.2017.04.010

33. Schwartz RA, Kapila R. Cutaneous manifestations of a 21st century worldwide fungal epidemic possibly complicating the COVID-19 pandemic to jointly menace mankind. *Dermatol Ther*. 2020;33(4):e13481. https://doi.org/10.1111/dth.134814of4

34. Bishburg E, Okoh A, Nagarakanti SR, Lindner M, Migliore C, Patel P. Fungemia in COVID-19 ICU patients, a single medical center experience. *J Med Virol*. 2020;93(5):2810-4. https://doi.org/10.1002/jmv.26633

35. Macauley P, Epelbaum O. Epidemiology and Mycology of Candidaemia in non-oncological medical intensive care unit patients in a tertiary center in the United States: overall analysis and comparison between non-COVID-19 and COVID-19 cases. *Mycoses*. 2021;64(6): 634-40. https://doi.org/10.1111/myc.13258

36. Seagle EE, Jackson BR, Lockhart SR, Georgacopoulos O, Nunnally NS, Roland J, et al. The landscape of candidemia during the COVID-19 pandemic. *Clin Infect Dis*. 2022;74(5):802-11. https://doi.org/10.1093/cid/ciab562

37. Oliveira CS, Colombo AL, Francisco EC, Lima B, Gandra RF, Carvalho MCP, et al. Clinical and epidemiological aspects of Candidemia in eight medical centers in the state of Parana, Brazil: Parana Candidemia Network. Braz J Infect Dis. 2021;25(1):101041 https://doi.org/10.1016/j.bjid.2020.11.006.

38. Chowdhary A, Tarai B, Singh A, Sharma A. Multidrug-resistant Candida auris infections in critically ill coronavirus disease patients. *Emerg Infect Dis*. 2020;26(11):2694-6. https://doi.org/10.3201/eid2611.203504

39. Magnasco L, Mikulska M, Giacobbe DR, Taramasso L, Vena A, Dentone C, et al. Spread of Carbapenem- resistant gram-negatives and Candida auris during the COVID-19 pandemic in critically ill patients: one step back in antimicrobial stewardship? *Microorganisms*. 2021;9(1):95. https://doi.org/10.3390/microorganisms9010095

40. Riche, CVW, Cassol R, Pasqualotto AC. Is the frequency of Candidemia increasing in COVID-19 patients receiving corticosteroids? *J Fungi*. 2020;6(4):286. https://doi.org/10.3390/jof6040286

41. Zoorob RJ, Cender D. A different look at corticosteroids. *Am Fam Physician*. 1998;58(2):443-50.

42. Cruz-Topete D, Cidlowski JA. One hormone, two actions: anti- and pro-inflammatory effects of glucocorticoids. Neuroimmunomodulation. 2015;22:20-32. https://doi.org/10.1159/000362724.

43. Noreen S, Maqbool I, Madni A. Dexamethasone: therapeutic potential, risks, and future projection during COVID-19 pandemic. *Eur J Pharmacol*. 2021;894:173854.https://doi.org/10.1016/j.ejphar.2021.173854

44. Shi W, Wang T, Xie L, Li S, Gao H, Liu J, et al. Risk factors, clinical features, and outcomes of recurrent fungal keratitis after corneal transplantation. *Ophthalmol*. 2010;117(5):890-6.

45. Beardsley J, Wolbers M, Kibengo FM, Ggayi ABM, Kamali A, Cuc NTK, et al. Adjunctive Dexamethasone in HIV-associated cryptococcal meningitis. *N Engl J Med*. 2016;374(6):543-54. https://doi.org/10.1056/nejmoa1509024

46. Knutsson KA, Iovieno A, Matuska S, Fontana L, Rama P. Topical corticosteroids and fungal keratitis: a review of the literature and case series. *J Clin Med*. 2021;10(6):1178. https://doi.org/10.3390/jcm10061178

47. Calderón-Parra J, Muiño-Miguez A, Bendala-Estrada AD, Ramos-Martínez A, Muñez-Rubio E, Carracedo EF, et al. Inappropriate antibiotic use in the COVID-19 era: factors associated with inappropriate prescribing and secondary complications: analysis of the registry SEMI-COVID. *PLoS One*. 2021;16(5):e0251340. https://doi.org/10.1371/journal.pone.0251340

48. Marcolino MS, Ziegelmann PK, Souza-Silva MVR, Nascimento IJB, Oliveira LM, Monteiro LS, et al.. Clinical characteristics and outcomes of patients hospitalized with COVID-19 in Brazil: results from the Brazilian COVID-19 registry. *Int J Infect Dis*. 2021;107:300-10. https://doi.org/10.1016/j.ijid.2021.01.019

49. Pemán J, Ruiz-Gaitán A, García-Vidal C, Salavert M, Ramírez P, Puchades F, et al. Fungal co-infection in COVID-19 patients: should we be concerned? *Rev Iberoam Micol*. 2020;37(2):41-46. https://doi.org/10.1016/j.riam.2020.07.001

50. Stratman RC, Martin CA, Rapp RP, Berger R, Magnuson B. Candidemia incidence in recipients of parenteral nutrition. *Nutr Clin Pract*. 2010;25(3):282-9. https://doi.org/10.1177/0884533610368704

51. Reginatto P, Bergamo VZ, Berlitz SJ, Guerreiro ICK, Andrade SF, Fuentefria AM. Rational selection of antifungal drugs to propose a new formulation strategy to control Candida biofilm formation on venous catheters. *Braz J Microbiol*. 2020;51(3):1037-49. https://doi.org/10.1007/s42770-020-00242-z

52. Chiurlo M, Mastrangelo A, Ripa M, Scarpellini P. Invasive fungal infections in patients with COVID-19: a review on pathogenesis, epidemiology, clinical features, treatment, and outcomes. *New Microbiol*. 2021;44(2):71-83.

53. Fram DS, Ferreira DB, Matias LO, Coelho WE, Escudero DV, Antonelli TS, et al. Perfil epidemiológico das iras notificadas em um hospital universitário durante a pandemia da covid-19. *Braz J Infect Dis*. 2021;25(Suppl 1):101063.

54. Azoulay E, Timsit JF, Tafflet M, Lassence A, Darmon M, Zahar JR, et al. Candida colonization of the respiratory tract and subsequent pseudomonas ventilator-associated pneumonia. *Chest*. 2006;129(1):110-7.

55. Lilienfeld-Toal M, Wagener J, Einsele H, Cornely OA, Kurzai O. Invasive fungal infection. Dtsch Arztebl Int. 2019;116(16):271-8.

56. Arastehfar A, Shaban T, Zarrinfar H.; Roudbary M, Ghazanfari M, Hedayati MT, et al. Candidemia among Iranian patients with severe COVID-19 admitted to ICUs. *J Fungi*. 2021;7(4):280. https://doi.org/ 10.3390/jof7040280

57. Kayaaslan B, Eser F, Kalem AK, Bilgic Z, Asilturk D, Hasanoglu I, et al. Characteristics of candidemia in COVID-19 patients; increased incidence, earlier occurrence and higher mortality rates compared to non-COVID-19 patient. *Mycoses*. 2021;64(9):1083-91. https://doi.org/10.1111/myc.13332

58. Abdullah AMS, Mohsin J, Al-Huraizi A, Khamis F. COVID-19 associated invasive candidiasis. *J Infect*. 2020;82(2):e45-46. https://doi.org/10.1016/j.jinf.2020.08.005

59. Mastrangelo A, Germinario BN, Ferrante M, Frangi C, Voti RL, Muccini C, et al. Candidemia in coronavirus disease 2019 (COVID-19) patients: incidence and characteristics in a prospective cohort compared to historical non-COVID-19 controls. *Clin Infect Dis*. 2020;73(9):e2838-9.

60. Giacobbe DR, Battaglini D, Ball L, Brunetti I, Bruzzone B, Codda G, et al. Bloodstream infections in critically ill patients with COVID-19. *Eur J Clin Invest*. 2020;50(10):e13319. https://doi.org/10.1111/eci.13319

61. White PL, Dhillon R, Cordey A, Hughes H, Faggian F, Soni S, et al. A national strategy to diagnose coronavirus disease 2019-associated invasive fungal disease in the Intensive Care Unit. *Clin Infect Dis*. 2021;73(7):e1634-44.

62. Agrifoglio A, Cachafeiro L, Figueira JC, Añón JM, Lorenzo AG. Critically ill patients with COVID-19 and candidaemia: we must keep this in mind. *J Mycol Med*. 2020;30(4):101012. https://doi.org/10.1016/j.mycmed.2020.101012

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HABILIDADES SOCIAIS EM PACIENTES EM UMA UNIDADE DE INTERNAÇÃO PSIQUIÁTRICA: UM ESTUDO TRANSVERSAL

SOCIAL SKILLS IN PATIENTS IN A PSYCHIATRIC INPATIENT UNIT: A CROSS-SECTIONAL STUDY

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RESUMO

Introdução: Pessoas com transtornos psiquiátricos tendem a apresentar déficit nas habilidades sociais. Este estudo busca mapear o nível de habilidades sociais, considerando questões básicas como iniciar e manter conversação e estabelecer e manter relações de amizade e românticas.

Métodos: Estudo transversal com 91 pacientes internados em unidade psiquiátrica de um hospital geral. Foi utilizado o item Habilidades Sociais do questionário elaborado pelos autores do projeto para obtenção de dados para análise descritiva e para avaliar associações entre características demográficas e percepção de prejuízo nas habilidades.

Resultados: 59,3% consideram que apresentam dificuldade grave em pelo menos 1 item e 21,9% em 3 ou mais itens. O item em que os participantes consideraram que possuem maiores dificuldades foi estabelecer relações românticas (41,8%), enquanto o item que consideraram ter maior facilidade foi comunicar-se com familiares em casa (79,1%). Status de relacionamento está estatisticamente relacionado com a autopercepção da capacidade de estabelecer relações românticas. Há uma tendência de que pessoas sem alguém tenham mais dificuldade em habilidades sociais e que mulheres avaliem terem mais dificuldades na habilidade de estabelecer relações românticas do que os homens.

Conclusões: Indivíduos em momento de agudização do sofrimento mental apresentam expressivo déficit nas habilidades sociais. O nível de prejuízo aumenta à medida que as interações se tornam mais complexas e mais distante do núcleo familiar. Assim, é importante que essas questões sejam tratadas desde a internação psiquiátrica.

Palavras chave: *Habilidades Sociais, Funcionamento Psicossocial, Transtornos Mentais, Saúde Mental, Adulto.*

ABSTRACT

Background: People with psychiatric disorders often have deficits in social skills. This study seeks to map the level of social skills, considering basic issues such as initiating and maintaining conversation and establishing and maintaining friendships and romantic relationships.

Methods: Cross-sectional study with 91 patients admitted to the psychiatric unit of a general hospital. The Social Skills subsection of the questionnaire prepared by the project authors was used to obtain data for descriptive analysis and verification of associations between demographic characteristics and perception of impairment in social skills.

Results: 59.3% consider that they have severe difficulty in at least 1 item and 21.9% in 3 or more items. The item in which the participants considered that they had

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greater difficulties was establishing romantic relationships (41.8%), while the item that they considered as having less difficulties was communicating with family members at home (79.1%). The relationship status was statistically related to the self-perceived ability to establish romantic relationships. There is a tendency for people without someone to have more difficulties with social skills and for women to rate themselves with more difficulties in the ability to establish romantic relationships than men.

Conclusions: Individuals in the stage of acute mental distress present a significant deficit in social skills. The level of impairment increases as interactions become more complex and less familiar. Thus, these issues should be addressed from the time of psychiatric hospitalization.

Keywords: *Social Skills, Psychosocial Functioning, Mental Disorders, Mental Health, Adult.*

INTRODUÇÃO

A Rede de Atenção Psicossocial (RAPS) instituída na Portaria 3088 de 2011 reconhece a criação, ampliação e articulação dos pontos de atenção à saúde de pessoas com sofrimento mental no âmbito do Sistema Único de Saúde (SUS). Um dos pontos essenciais para o funcionamento desta rede são as enfermarias especializadas em saúde mental alojadas nos hospitais gerais e que ofertam tratamento visando minimizar riscos e tratar prejuízos funcionais decorrentes do agravamento e agudização da condição dos usuários. O cuidado ofertado deve ser de curta duração, multidisciplinar e estar articulado com o Projeto Terapêutico Individual desenvolvido pelo serviço de referência do usuário[1].

Além do tratamento biomédico, que visa o esbatimento dos sintomas, em uma internação psiquiátrica, é necessário que os trabalhadores da saúde busquem compreender o sujeito em sua singularidade. Deve ser assegurada a assistência médica, mas também psicológica, social, ocupacional e de lazer, de acordo com as demandas individuais e integrais dos pacientes[2].

De acordo com as recomendações farmacológicas do Schizophrenia Patient Outcomes Research Team (PORT)[3], uma porcentagem significativa de pessoas com esquizofrenia mantém os sintomas negativos, como isolacionismo, embotamento afetivo, pobreza do discurso, bradicinesia e baixa funcionalidade, apesar de diferentes esquemas medicamentosos. Os autores destacam que estes sintomas representam uma necessidade de tratamento ainda não atendida.

Atualmente, há uma tendência na mudança de foco dos serviços: em vez de focar nas categorias diagnósticas, priorizam parâmetros funcionais, com impacto na qualidade de vida e inclusão do indivíduo – os chamados recovery-oriented services (ou serviços focados na recuperação/reabilitação)[4,5]. Nesse âmbito, autores[6,7] ressaltam as necessidades sociais de indivíduos com transtorno mental, comentando sobre sentimento de solidão e desejo por amizades.

Habilidades sociais são capacidades comportamentais aprendidas que auxiliam as pessoas a expressar seus sentimentos e estabelecer relacionamentos, tanto no âmbito social, como familiar e profissional. Déficits nas habilidades sociais são comuns em pessoas com transtornos de personalidade, transtornos de ansiedade, esquizofrenia, abuso de álcool e outras drogas, entre outros[8,9]. Essas habilidades podem estar ainda mais prejudicadas quando da agudização dos sintomas[10], quando, por vezes, ocorre a indicação de internação psiquiátrica.

Este estudo busca mapear o nível de habilidades sociais, considerando questões básicas como iniciar e manter conversação e estabelecer e manter relações de amizade e românticas, de pacientes internados em uma unidade psiquiátrica de um hospital geral. Além disso, buscou-se analisar associações entre percepção do desempenho nas habilidades sociais e variáveis demográficas. O conhecimento das habilidades e necessidades da clientela é o 1º passo para elaboração de intervenções específicas que visem o desenvolvimento pessoal e a melhora na qualidade de vida - aspectos essenciais em um tratamento que considere o indivíduo em sua integralidade.

MÉTODO

Trata-se de uma pesquisa descritiva, quantitativa, com delineamento transversal, com pacientes internados na Unidade de Internação Psiquiátrica Adulto do Hospital de Clínicas de Porto Alegre (HCPA), Rio Grande do Sul. A unidade atende situações psiquiátricas agudas graves, geralmente associadas a riscos de auto e heteroagressão, tentativas de suicídio e/ou confusão mental. É composta por 35 leitos, sendo 26 conveniados pelo SUS e 9 direcionados para internações particulares ou convênios privados. Os leitos SUS são regulados pelo sistema informatizado de Gerenciamento de Internações (GERINT) e atende a pacientes provenientes das redes de Urgência e Emergência em Saúde Mental do município.

Esta é uma amostra por conveniência, selecionada a partir do grupo de pacientes que internaram na unidade psiquiátrica no período de 01 de agosto a 31 de dezembro de 2021, pelo GERINT - ou seja, leitos conveniados ao SUS, o que totalizou 146 pacientes. Noventa e um pacientes compuseram a amostra final. O fluxograma (Figura 1) especifica as razões para a não inclusão dos demais.

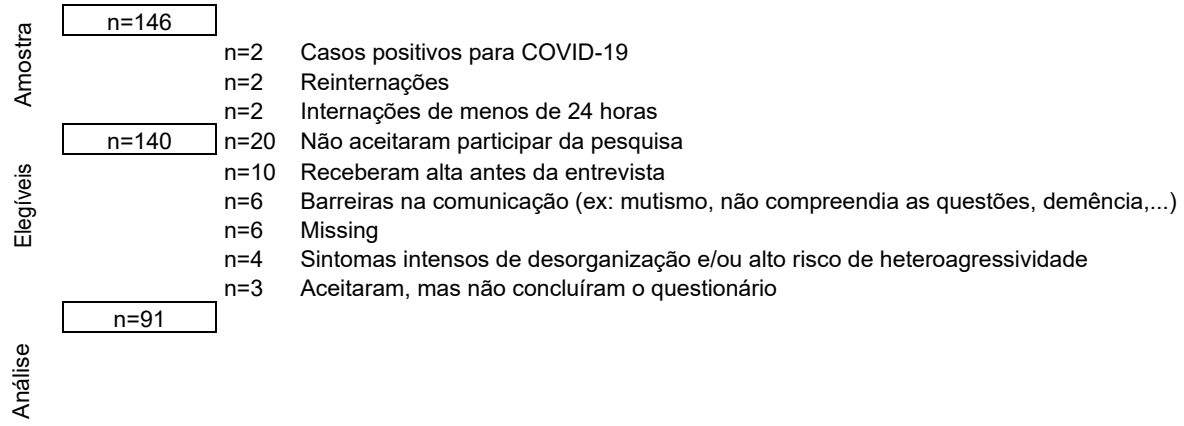

Figura 1: Fluxograma do processo de seleção da amostra.

Em um período máximo de 48 horas após a internação, os pacientes foram abordados e convidados a participar da pesquisa. Após o consentimento verbal, o paciente era encaminhado para um consultório privativo, quando o Termo de Consentimento Livre e Esclarecido (TCLE) era lido e explicado. Frente a assinatura do termo, era realizado um questionário, elaborado pelos autores e baseado na vivência prática destes enquanto trabalhadores da saúde mental. O questionário era dividido em duas partes: características sociodemográficas e Escala de Avaliação Psicossocial – esta formada por 23 perguntas fechadas e divididas em quatro domínios independentes: Funcionalidade, Autocuidado, Saúde Mental e Habilidade Sociais. O questionário completo não foi publicado, sendo trabalhado cada domínio de forma separada e independente.

O domínio das Habilidades Sociais - foco deste trabalho - possui cinco questões que abrangem a autopercepção da capacidade de comunicar-se em casa, de participar de eventos sociais, de iniciar conversação em ambientes familiares e de estabelecer relações de amizade e românticas. As questões eram ranqueadas em uma escala likert de 5 pontos, sendo *consegue com facilidade* o extremo positivo de desempenho e *não consegue* o extremo oposto.

Os dados enviados em programa Excel foram transpostos para o Statistical Package for Social Science for Windows (SPSS) versão 29.0. Realizou-se análise descritiva dos dados para caracterização da amostra e panorama geral das respostas. Utilizou-se o teste qui-quadrado de Pearson com correção de Yates ou teste exato de Fisher (quando necessário) para avaliar associações entre características demográficas e percepção de prejuízo de dificuldades. Foi considerado um nível de significância de $\alpha = 0,05$.

O projeto, registrado na Plataforma Brasil sob o número CAEE 07515018.5.0000.5327, foi aprovado pelo Comitê de Ética em Pesquisa da instituição na qual foi desenvolvido através do parecer número 4.786.339. Este estudo foi projetado conforme as Diretrizes e Normas Regulamentadoras de Pesquisas Envolvendo Seres Humanos de acordo com a Resolução do Conselho Nacional de Saúde nº466 de 2012.1. Os pesquisadores assinaram a "Declaração de Cumprimento da LGPD".

RESULTADOS

Um total de 91 pacientes completaram o questionário e formaram a amostra deste estudo. Os dados demográficos estão descritos na Tabela 1. A população amostral tinha idade entre 18 e 84 anos, com média de idade de 44,35 anos (dp=16,82) e mediana de 44 anos. A maioria dos pacientes era solteira, do sexo feminino, branca e procedente de Porto Alegre. A escolaridade concentrou-se em Ensino Fundamental incompleto e Ensino Médio completo. A maioria dos pacientes mora em Porto Alegre e não reside sozinho. Cerca de metade recebe algum tipo de benefício. O tempo de internação variou entre 03 e 121 dias, com média de 23,19 dias (dp=19,27) e mediana de 17,07 dias.

Quase 60% (59,3%) da amostra consideram que apresentam dificuldade grave em pelo menos 1 item e 21,9% se percebem com dificuldade grave em 3 ou mais itens. O item em que os participantes consideram que possuem maiores dificuldades foi estabelecer relações românticas (41,8%), enquanto o item em que os participantes consideram ter maior facilidade foi comunicar-se com familiares em casa (79,1%). A Tabela 2 apresenta os resultados referente ao nível de percepção das habilidades sociais, conforme a Escala de Avaliação Psicossocial.

Tabela 1: Características sociodemográficas da amostra.

Idade	**n(%)**
18-29 anos	20 (21,98)
30-59 anos	56 (61,54)
60-84 anos	15 (16,48)
Sexo	
Feminino	48 (52,7)
Masculino	43 (47,3)
Etnia/cor	
Branca	51 (56,0)
Parda/Preta	35 (38,5)
Outras (Indígena, Amarela)	5 (5,5)
Estado Civil	
Solteiro	54 (59,3)
Casado	20 (22,0)
Divorciado, desquitado ou separado	12 (13,2)
Viúvo	4 (4,4)
Status do relacionamento	
Solteiro	42 (46,2)
Casado	26 (28,6)
Sem relacionamentos	12 (13,2)
Namorando	7 (7,7)
Morando junto	3 (3,3)
Viúvo	1 (1,1)
Cidade origem	
Porto Alegre	56 (61,5)
Região metropolitana	7 (7,7)
Interior do RS	23 (25,3)
Outros	5 (5,5)
Situação de moradia	
Mora com familiares/amigos em casa/apartamento	67 (73,6)
Mora sozinho em casa/apartamento	19 (20,9)
Residencial Terapêutico	2 (2,2)
Abrigo	1 (1,1)
Situação de rua	1 (1,1)
Escolaridade	
Nunca frequentou	1 (1,1)
Ensino Fundamental incompleto	28 (30,8)
Ensino Fundamental (completo)	22 (23,2)
Ensino Médio (completo)	23 (25,3)
Ensino Superior incompleto	10 (11,0)

Continua...

Tabela 1: Continuação

Especialização ou Ensino Superior (completo)	7 (7,7)
Situação de trabalho	
Desempregado	63 (69,2)
Emprego Formal	18 (19,8)
Emprego Informal	9 (9,9)
Benefício	
Sim	45 (49,5)
Não	46 (50,5)
Benefício (tipo)	
Aposentadoria	21 (23,1)
Bolsa Família	7 (7,7)
Benefício de Prestação Continuada (BPC)	6 (6,6)
Auxílio Emergencial	5 (5,5)
Seguro Desemprego	2 (2,2)
Outros	4 (4,4)
Tempo de internação	
Até 21 dias	58 (63,74)
Entre 21 e 42 dias	20 (21,98)
Mais de 42 dias	13 (14,29)

Fonte: Escala de Avaliação Psicossocial.

Tabela 2: Habilidades sociais: capacidades conforme autoavaliação no questionário desenvolvido pelas pesquisadoras (n = 91).

	Facilidade ou pouca dificuldade	**Dificuldades moderadas**	**Dificuldades graves/não consegue**
Comunica-se com familiares em casa	72(79,1)	10(11)	9(9,9)
Participa de eventos sociais	56(61,5)	9(9,9)	25(27,5)
Iniciar conversação em ambientes familiares	61(67)	6(6,6)	23(25,3)
Estabelecer relações de amizade	61(67)	9(9,9)	21(23,1)
Estabelecer relações românticas	44(48,4)	8(8,8)	38(41,8)

Fonte: Escala de Avaliação Psicossocial.

Em uma exploração adicional dos dados, buscou verificar correlações entre as variáveis demográficas e os cinco itens que compõem o domínio habilidades sociais. Utilizando o teste Chi Quadrado, não houve diferença estatisticamente significativa entre os itens do questionário aplicado e as variáveis idade, emprego, se recebe benefício e tempo de internação.

O item status de relacionamento está estatisticamente relacionado com a auto percepção da capacidade de estabelecer relações românticas (<0.001 - teste de exato de Fisher). Para essa análise, os dados foram agrupados em dois grupos: *com alguém* (unindo os dados dos que se identificam: namorando, morando junto e casado) e *sem alguém* (subgrupo advindo da união dos que se identificam como: solteiro, sem relacionamento e viúvo). Mais de 70% das pessoas do grupo *com alguém* tem pouca ou nenhuma dificuldade em estabelecer relações românticas, enquanto que este percentual cai para 33,3% na categoria *sem alguém.* Ainda comparando estes dois grupos, há uma tendência de que pessoas *sem*

alguém tenham mais dificuldade em habilidades sociais em geral (compilando os 5 itens do questionário), com p=0,064. Das 55 pessoas sem alguém, 76,4% apresenta dificuldade moderada ou grave em 3 ou mais itens; já entre as 36 pessoas com alguém, este índice cai para 55,6%. Acredita-se que, aumentando o n da amostra, haveria significância na correlação entre estas variáveis.

Apesar de não ter sido estatisticamente significativa, há indício de correlação (p=0,094) entre sexo e capacidade de estabelecer relações românticas, com tendência a mulheres se avaliarem com mais dificuldades: 60,4% das mulheres avaliam-se como tendo dificuldades moderada ou grave neste quesito, enquanto este índice corresponde a 40,5% dos homens (conforme Tabela 3).

Tabela 3: Habilidade social no item relações românticas por sexo e status de relacionamento (n = 91).

Status de relacionamento	Facilidade ou pouca dificuldade	Dificuldades moderadas ou grave	Valor P*
			<0,001
Com alguém	26(72,2)	10(27,8)	
Sem alguém	18(33,3)	36(66,7)	
Sexo			0,094
Homens	25(59,5)	17(40,5)	
Mulheres	19(39,6)	29(60,4)	

Fonte: Escala de Avaliação Psicossocial. *Teste de qui-quadrado de Pearson.

DISCUSSÃO

Através da análise dos dados, percebe-se uma importante dificuldade no âmbito das habilidades sociais. Entre os domínios avaliados, a habilidade de comunicar-se com familiares é a mais desenvolvida: pouco mais de 20% refere possuir dificuldade moderada ou grave. Entretanto, quando a pergunta exige uma postura mais ativa, como iniciar uma conversação, mesmo que em ambiente familiar, este índice percentual vai para 39% de usuários que apresentam dificuldade moderada ou grave. O mesmo valor é encontrado quando avaliamos a capacidade de estabelecer relações de amizade. A taxa de 44% de pessoas que consideram que possuem dificuldade moderada ou grave na capacidade de participar de eventos demonstra uma tendência no aumento da percepção de dificuldades à medida em que há um incremento da complexidade de relações fora do âmbito familiar. Mais da metade da amostra (56%) considera que possui dificuldades moderadas ou graves para estabelecer relações românticas. No cruzamento dos dados do questionário com as variáveis sóciodemográficas, estar sem alguém foi correlacionado à percepção de maior dificuldade em estabelecer relações românticas, e apresentou uma tendência a maiores dificuldades em habilidades sociais no geral.

As dificuldades nas habilidades sociais encontradas neste estudo são corroboradas por outras pesquisas, como a realizada com 380 pacientes internados em hospital psiquiátrico, na qual avaliou-se habilidades sociais em três áreas: comportamento social, comportamento verbal e comportamento não verbal. A maior dificuldade dos participantes era em se voluntariar para ajudar outras pessoas (26,3%), seguida pela dificuldade em compreender a perspectiva do outro (25,8%) e de expressar sua própria emoção ou experiência (23,0%). Na área do comportamento verbal, a maior dificuldade foi para iniciar e se engajar em conversação (24.3%)[4].

Outros autores[7,11,12] ressaltam o prejuízo que o deficit nas habilidades sociais causam nos diversos âmbitos da vida real dos indivíduos com transtornos mentais. Estudos internacionais[11,13,14] mostram a importância e o impacto de programas que consideram o indivíduo em sua integralidade, com intervenções não apenas farmacológicas, mas também de reabilitação psicossocial desde a internação psiquiátrica. Apesar disso, não foram encontrados estudos no Brasil que tenham avaliado intervenções como estas na nossa população - nem mesmo que tenham medido essas habilidades nestes pacientes em momento de crise.

A manutenção de relações interpessoais favorece o processo de inserção e atuação em sociedade, tanto pela satisfação advinda deste vínculo, quanto pela habilidade social necessária para a própria convivência, o que reflete no refinamento de comportamento indispensável para qualquer interação social. Os vínculos de qualidade se iniciam devido habilidades sociais prévias, mas são mantidos pela própria manutenção das relações, as quais permitem o desenvolvimento das habilidades sociais do indivíduo que acaba angariando apoio emocional, informacional, instrumental para amenizar e solucionar dificuldades[15]. As habilidades sociais podem ser adquiridas e refinadas ao longo da vida:

o bom desenvolvimento de uma rede social de suporte pode auxiliar nas demandas de vida do indivíduo[16].

Estudos conduzidos com adolescentes com depressão apontam para um melhor desempenho dos meninos em relação às meninas nos indicadores de empatia, autocontrole, civilidade e assertividade. Ainda que, de modo geral, tanto meninos quanto meninas diagnosticados com depressão apresentam piora no desempenho das habilidades sociais, a queda é mais acentuada na amostra das meninas[16]. Apesar do estudo[16] tratar de adolescentes, está de acordo com os resultados apresentados por esta pesquisa, que aponta que, apesar de não estatisticamente significativo, as mulheres apresentaram tendência a se perceberem com mais dificuldade do que os homens.

Enfim, este estudo visou mapear o nível de habilidades sociais de pacientes internados em uma unidade psiquiátrica. Apesar do estudo ter sido conduzido em apenas um centro e se basear na autopercepção dos indivíduos, permitiu lançar luz sobre aspectos pouco vistos quando em momento de crise: Cerca de 25% dos pacientes internados em unidade psiquiátrica possuem dificuldade de iniciar conversação, estabelecer relações de amizade e participar de eventos sociais e mais de 40% da amostra apresentou dificuldade para estabelecer relações românticas. O prejuízo nas habilidades sociais se agrava à medida que o indivíduo precisa interagir com grupos de pessoas cada vez mais distantes do convívio diário.

O prejuízo nas habilidades sociais pode ser considerado um fator estressor e de risco para desencadear transtornos mentais, um sintoma dos transtornos mentais, ou até mesmo um fruto da progressão dos transtornos mentais. De qualquer forma, são habilidades fundamentais para uma vida plena e com significado, considerando o indivíduo em sua integralidade. Considerando a internação psiquiátrica o local adequado para o tratamento quando há agravamento e agudização do quadro psiquiátrico, deve-se buscar intervenções que busquem minimizar riscos e melhorar prejuízos funcionais. Uma unidade de internação psiquiátrica que realiza apenas tratamento medicamentoso torna-se apenas um local de contenção, deixando de abarcar aspectos subjetivos e de vida do indivíduo. Muitas vezes, são estes que precisam ser ativados para que o tratamento faça sentido e, assim, motive os pacientes a aderir à terapêutica proposta.

Assim, destaca-se que o mapeamento é o primeiro passo para que se tenha contato e dimensão da problemática. Independente do deficit em habilidades sociais ser desencadeador ou consequência do agravamento da condição psiquiátrica, ressalta-se a importância de que seja olhado e reconhecido durante o momento da internação psiquiátrica para que se possa iniciar o tratamento a curto prazo e pensar em estratégias terapêuticas a longo prazo.

Considerando a amostra reduzida e o fato da pesquisa ter sido conduzida em um único centro, um hospital geral de alta complexidade, considera-se relevante a replicação deste estudo. Dados de outros centros, que atendam o mesmo público, tendem a dar mais robustez aos resultados. O uso de um questionário simples, que considera e valoriza a percepção do indivíduo quanto a si mesmo, facilita a utilização em diferentes contextos. A possibilidade de reaplicação permite avaliar avanços e/ou retrocessos no tratamento. Por fim, o fato de ser baseado na autopercepção tende a implicar o usuário a se envolver nos objetivos do tratamento.

REFERÊNCIAS

1. Ministério da Saúde (Brasil). Portaria nº 3.088, de 23 de dezembro de 2011. Institui a Rede de Atenção Psicossocial para pessoas com sofrimento ou transtorno mental e com necessidades decorrentes do uso de crack, álcool e outras drogas, no âmbito do Sistema Único de Saúde (SUS). *Diário Oficial da União*, 30 dez 2011, Seção 1.

2. Del'Olmo FS, Cervi TMD. Sofrimento mental e dignidade da pessoa humana: os desafios da reforma psiquiátrica no Brasil. *Seqüência*. 2017;38(77):197-220.

3. Buchanan RW, Kreyenbuhl J, Kelly DL, Noel JM, Boggs DL, Fischer BA, et al. The 2009 schizophrenia PORT psychopharmacological treatment recommendations and summary statements. *Schizophr Bull*. 2010;36(1):71-93.

4. Bhola P, Basavarajappa C, Guruprasad D, Hegde G, Khanam F, Thirthalli J, et al. Development of a social skills assessment screening scale for psychiatric rehabilitation settings: a pilot study. *Indian J Psychol Med*. 2016;38(5):395-403.

5. Killaspy H, King M, Holloway F, Craig TJ, Cook S, Mundy T, et al. The Rehabilitation Effectiveness for Activities for Life (REAL) study: a national programme of research into NHS inpatient mental health rehabilitation services across England. *Programme Grants Appl Res*. 2017;5(7).

6. Stain HJ, Galletly CA, Clark S, Wilson J, Killen EA, Anthes L, et al. Understanding the social costs of psychosis: the experience of adults affected by psychosis identified within the second Australian National Survey of Psychosis. *Aust N Z J Psychiatry*. 2012;46(9):879-889.

7. Taylor R, Cella M, Csipke E, Heriot-Maitland C, Gibbs C, Wykes T. Tackling social cognition in schizophrenia: a randomized feasibility trial. *Behav Cogn Psychother*. 2016;44(3):306-17.

8. Biasotto FF. Habilidades sociais e sofrimento psicológico. *Arq bras psicol*. 2013;65(1):38-50.

9. Sadock BJ, Sadock VA, Ruiz P. Compêndio de psiquiatria: ciência do comportamento e psiquiatria clínica. 11. ed. Porto Alegre (RS): Artmed; 2017.

10. Zanardo GLP, Silveira LHP, Rocha CMF, Rocha KB. Internações e reinternações psiquiátricas em um hospital geral de Porto Alegre: características sociodemográficas, clínicas e do uso da Rede de Atenção Psicossocial. *Rev bras epidemiol*. 2017;20(3):460-74.

11. Moe AM, Pine JG, Weiss DM, Wilson AC, Stewart AM, McDonald M, et al. A pilot study of a brief inpatient social-skills training for young adults with psychosis. *Psychiatr Rehabil J*. 2021;44(3):284-90.

12. Stålberg G, Ekselius L, Lindström LH, Larhammar D, Bodén R. Neuropeptide Y, social function and long-term outcome in schizophrenia. *Schizophr Res*. 2014;156(2-3):223-7.

13. Maxwell A, Tsoutsoulis K, Padinjareveettil AMT, Zivkovic F, Rogers JM. Longitudinal analysis of statistical and clinically significant psychosocial change following mental health rehabilitation. *Disabil Rehabil*. 2019;41(24):2927-39.

14. Sánchez P, Peña J, Bengoetxea E, Ojeda N, Elizagárate E, Ezcurra J, et al. Improvements in negative symptoms and functional outcome after a new generation cognitive remediation program: a randomized controlled trial. *Schizophr Bull*. 2014;40(3):707-15.

15. Ximenes VS, Queluz FNFR, Barham EJ. Revisão sistemática sobre fatores associados à relação entre habilidades sociais e suporte social. *Psico*. 2019;50(3),e31349.

16. Campos JR, Prette ZAPD, Prette AD. Relações entre depressão, habilidades sociais, sexo e nível socioeconômico em grandes amostras de adolescentes. Psic: Teor Pesq. 2018;34:e3446.

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Perinatal outcomes in a hospital from southern Brazil: Predictors of low APGAR in the first minute of birth

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ABSTRACT

Introduction: The Apgar Index (AI), is a method that assesses the physical conditions of the newborn (NB) in the period immediately after birth. The index proved to be a predictor of neonatal mortality in several clinical and population-based studies and has been used as a health assessment parameter in the immediate neonatal period for over 60 years.

Objective: To evaluate obstetric history and perinatal outcomes in newborns (NB) Apgar score equal zero in the first minute of life. Data from births in a public hospital in southern Brazil were evaluated. The studied population was divided into two groups: cases (AI equal zero) and controls (NB with AI ≥8). The cases were composed of 219 NB and the controls of 657 NB.

Results: In the multivariate analysis, the maternal variables predictors of AI zero in the first minute of life were: mother's age >25 years [odds ratio (OR): 1.8; confidence interval (95%CI): 1.1 – 2.8; p = 0.020] and absence of prenatal care (OR: 2.8; 95%CI: 1.5 – 5.5; p = 0.010). Death in the delivery room was higher in cases compared to controls ($p < 0.001$). Additionally, predictive neonatal variables were: umbilical arterial pH (<7.0) (OR: 5.2; 95%CI: 4.0 – 8.8; p <0.001), base excess (>-6.5) (OR: 4.1; 95%CI: 3.3 – 10.5; p <0.001), fetal breech presentation (OR: 7.1; 95%CI: 4.2 – 13.1; p <0.001), gestational age <33 weeks (OR: 15.1; 95%CI: 5.8 – 20.3; p <0.001), fetal weight <2500g (OR: 21.5; 95%CI: 12.1 – 31.9; p <0.001) and hemorrhagic amniotic fluid (OR: 13.8; 95%CI: 5.4 – 48.6; p <0.001).

Conclusion: Absence of prenatal care, maternal age, low birth weight, prematurity, breech presentation, hemorrhagic amniotic fluid, low AI in the fifth minute of life, lower umbilical artery pH values, and higher base excess values are related to AI zero in the first minute.

***Keywords**: Apgar score, Apgar zero first minute, perinatal outcomes.*

INTRODUCTION

The Apgar Index (AI), created by Virginia Apgar in 1953, is a method that assesses the physical conditions of the newborn (NB) in the period immediately after birth[1,2]. The index proved to be a predictor of neonatal mortality in several clinical and population-based studies and has been used as a health assessment parameter in the immediate neonatal period for over 60 years[1,3]. Its action as a predictor of the need for resuscitation and reassessment led it to be incorporated into various protocols and in the health policy of the World Health Organization (WHO)[3]. The index ranges from 0 to 10 and is measured at the 1st and 5th minute postpartum, and can also be measured at the 10th minute[4,5]. It is known that an AI of zero (apparently dead fetuses) is indicative of mortality and, especially, severe neonatal morbidity[1]. The index assesses muscle tone, breathing, heart rate, reflex activity, irritability, and skin color, all to classify the degree of fetal asphyxia as absent (8-10), mild (5-7), moderate (3-4), or low (0-2)[6]. Each criterion receives an evaluation of 0-2 points, according to the evaluation of the professional who assists the NB[5-7].

In recent years, there are few studies related to the epidemiological analysis of neonates with AI zero in the 1st minute of life, as well as their associations with

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obstetric and intrapartum history[1,8,9]. The frequency of births with AI zero ranged from 0.4 to 0.8/1,000 live births, and among these, mortality ranged from 28% to 57%. Survivors (14% to 60%) have neurological damage[9].

It is known that AI alone may not be related to fetal acidosis, especially in premature fetuses, as it is partially dependent on the maturity of the conceptus[10-13]. There are also few studies relating an AI of zero, or even less than five, with neonatal outcomes, as well as with maternal risk factors, labor, and mode of childbirth[1,9,14]. Other factors such as AI less than five in the 5th minute, time to subsequent resuscitation, time to reach a score equal to or greater than seven (time greater than ten minutes), and presence of seizure events in the first minutes of life are also associated with a worse prognosis. and a high neonatal mortality rate[1].

Despite the few and scarce studies in this area, it is known that NBs that are stillborn (AI zero in the 1st minute) are at greater risk for early death and adverse neurological outcomes, higher rates of resuscitation in the 1st minute, prematurity associated with low birth weight, fetal acidosis status, neurological impairments, and early mortality rate[3,6,9]. In addition, newborns who survive birth may develop neurological complications, namely epilepsy, cerebral palsy, and developmental delay[3,9]. The risk factors for perinatal asphyxia can be divided into antepartum, intrapartum, and fetal asphyxia, namely: pregnancy at the reproductive extremes of life, prolonged membrane rupture time, meconium amniotic fluid, absence of prenatal care, low birth weight, Antepartum hemorrhage, and anomalous presentations are risk factors related to low AI, consequent neonatal anoxia and need for treatment in a neonatal intensive care setting[3].

Given the importance of the topic and the scarcity of studies related to obstetric and perinatal history in cases of zero AI in the first minute, this case/control study was proposed at the Department of Gynecology and Obstetrics/Neonatology of a public hospital in southern Brazil.

METHODS

Study design and ethical issues

This is a case/control study nested within a cross-sectional study that analyzed, from May 1998 to December 2015, data from newborns from the Gynecology/Obstetrics and Neonatology Service of a public hospital in southern Brazil. The present study was approved by the Research Ethics Committee (protocol: 1.453.509).

Population studied

The population studied was divided into two groups:

Two groups were composed: Cases with 219 NB with AI equal zero in the first minute and controls with 657 NB (1:3 proportion about the number of cases) with AI ≥ 8.

Cases: where all births registered in the aforementioned period and which obtained AI equal zero in the first minute of life were included.

Controls: where the three NB with AI equal to or ≥8 in the first minute of life were randomly included in the same database, in a proportion of 3:1.

Inclusion criteria

All single pregnancies, with gestational age (GA) equal to or greater than 22 weeks or weight equal to or ≥500g, whose birth took place at a public hospital in southern Brazil were considered.

Exclusion criteria

Twin pregnancies, NB with GA less than 22 weeks or weight <500g, carriers of congenital anomalies, NB born outside the GH, and all medical records are incomplete or with inconsistent data.

Outcomes

Maternal: age (average); schooling (≥8 and <8 years); skin color (white and non-white); parity (primigravid and ≥2); weight at the beginning of pregnancy; tobacco and alcohol users; provision of prenatal care, mode of childbirth (vaginal and cesarean); type of amniotic fluid (meconium, hemorrhagic, or clear); hypertensive syndrome (composed of chronic hypertension, gestational hypertension, preeclampsia, preeclampsia superimposed on chronic hypertension, HELLP syndrome, and eclampsia) and diabetic syndrome (types 1 and 2 diabetes mellitus and gestational diabetes mellitus).

Neonatal: gestational age (mean, based on the date of last menstrual period and confirmed by early ultrasound or Capurro's method); fetal presentation (pelvic or cephalic); birth weight (500g up to 2500g and over 2,500g); umbilical artery pH and base excess; the need for treatment in a neonatal intensive care environment; AI in the fifth minute (median); early neonatal mortality (≥7 days); conditions of the newborns at the time of hospital discharge (death or discharge alive).

Statistical analysis

Data were analyzed using IBM SPSS® software, version 25.0 (Chicago, IL Statistical Package for the Social Sciences). For continuous data, normality was assessed using the Kolmogorov-Smirnov test, with Lilliefors correction. Student's t-test for independence samples was used to assess possible differences between the mean values in the study groups. Bivariate analyses were performed to assess the association between categorical variables, using Fisher's exact test or the chi-square test (as appropriate). The odds ratio (OR), as well as the respective 95% confidence interval

(95%CI), absolute and relative frequencies, were estimated for the categorical data using Pearson's chi-square test or Fisher's exact test. Multivariate analysis was performed by logistic regression. *P*-values <0.05 were considered statistically significant.

RESULTS

Table 1 presents the maternal variables analyzed in the study and is representative of the sample. The mean maternal age in cases was 26.8 ± 7.5 years and in controls, 25.3 ± 6.9 (p = 0.005). In the case group, the percentage of patients who had hypertensive syndrome was slightly higher than in the control group, with [14% (n=31) vs. 12% (n=81)], but without statistical significance. Regarding diabetic syndrome, the percentage of cases was lower than in controls [3% (n=7) vs. 7.5%(n=49); p = 0.038]. Most patients in both groups reported adequate prenatal care, although the percentage of patients without assistance was higher in cases [9.5% (n=20) vs. 3.4% (n=22); p <0.001]. The other variables did not show statistical significance.

Table 1: Distribution of maternal variables related to NBs with AI zero in the first minute in a case/control study of the Gynecology/Obstetrics and Neonatology Service of a public hospital in southern Brazil.

Variables	Cases (n=219)	Controls (n=657)	OR	CI95%	*p-values*
	n (%)	n (%)			
Age (mean ± SD)	26.8 ± 7.5	25.3 ± 6.9	-	-	**0.005**
Maternal weight (mean ± SD)	65.7 ± 15.6	66.2 ± 15.8	-	-	0.790
Years of schooling					
<8	49 (24.0)	210 (44.0)	0.39	0.27-0.57	**<0.001**
>8	158 (76.0)	267 (56.0)	1.0 (ref.)		
Self-declared skin color					
Not white	48 (24.5)	168 (26.0)	0.93	0.64-1.35	0.719
White	148 (75.5)	484 (74.0)	1.0 (ref.)		
Hypertensive syndrome					
Yes	31 (14.0)	81 (12.0)	1.17	0.75-1.81	0.559
No	188 (86.0)	576 (88.0)	1.0 (ref.)		
Diabetic syndrome					
Yes	7 (3.0)	49 (7.5)	0.41	0.18-0.91	**0.038**
No	212 (97.0)	608 (92.5)	1.0 (ref.)		
Prenatal					
No	20 (9.5)	22 (3.4)	3.2	1.62-5.67	**<0.001**
Yes	190 (90.5)	633 (96.6)	1.0 (ref.)		
Parity					
1	139 (64.0)	450 (68.5)	1.05	0.77-1.44	0.735
>2	79 (36.0)	207 (31.5)	1.0 (ref.)		
Smoking					
Yes	13 (24.0)	47 (17.0)	1.53	0.76-3.08	0.308
No	42 (76.0)	236 (83.0)	1.0 (ref.)		
Alcohol consumption					
Yes	3 (5.5)	4 (1.4)	3.98	0.86-18.31	0.090
No	52 (94.5)	276 (98.6)	1.0 (ref.)		

SD: Standard deviation; Ref.: reference category; Hypertensive syndrome (composed of chronic hypertension, gestational hypertension, preeclampsia, preeclampsia superimposed on chronic hypertension, HELLP syndrome, and eclampsia) and diabetic syndrome (types 1 and 2 diabetes mellitus and gestational diabetes mellitus); In bold, significant *p*-values are highlighted.

Table 2 presents the neonatal variables. In a comparison between cases and controls it was observed that: gestational age (in weeks) stands out (31.7 ± 5.8 vs. 38.7 ± 2.5; p <0.001), lower fetal weight at birth [(n=132 (75%) vs. n =12 (77); p <0.001], lower rates of umbilical artery pH (6.99 ± 0.22 vs. 7.25 ± 0.77; p <0.001), higher rates of base excess (-16.70 vs. -6.30; p <0.001), breech presentation [24% (n=44) vs. 4% (n=25); p <0.001] and a higher incidence of bleeding amniotic fluid during amniotomy [n=18 (9.1%) vs. n=4 (0.6%); p <0.001]. The mode of childbirth was similar in

both groups, with a majority of abdominal c-section delivery in both groups, although without statistical significance. The frequency of patients admitted to the ICU, the nursery evolution of early neonatal death, and death in the delivery room were higher in cases compared to controls ($p < 0.001$). In addition, the APGAR score at 5 minutes was significantly lower in cases compared to controls ($p < 0.001$).

Table 2: Distribution of variables related to newborns with Apgar zero in the first minute of life in a case/control study carried out at the Gynecology/Obstetrics and Neonatology Service of a public hospital in southern Brazil.

Variables	Cases (n=219)	Controls (n=657)	OR	CI95%	*p-values*
	n (%)	n (%)			
Umbilical arterial pH (mean ± SD)	6.99 ± 0.22	7.25 ± 0.77	-	-	**<0.001**
Base excess (mmol/L) (mean ± SD)	-16.7 ± 7.6	-6.3 ± 3.3	-	-	**<0.001**
Type of childbirth					
Cesarean	146 (72.0)	440 (77.0)	0.9	0.5-1.1	0.186
Normal	56 (28.0)	132 (23.0)	1.0 (ref.)		
Fetal presentation					
Pelvic	44 (24.0)	25 (4.0)	7.9	4.6-13.4	**<0.001**
Cephalic	140 (76.0)	600 (96.0)	1.0 (ref.)		
Gestacional age (mean ± SD)	31.7 ± 5.8	38.7 ± 2.5			**<0.001**
Fetal weight (g)					
500-2.499	132 (75.0)	77 (12.0)	22.6	14.9-34.2	**<0.001**
≥2.500	44 (25.0)	579 (88.0)	1.0 (ref.)		
Sex					
Male	23 (51.0)	149 (53.0)	0.9	0.5-1.8	0.956
Female	22 (49.0)	133 (47.0)	1.0 (ref.)		
Amniotic fluid					
Hemorrhagic	18 (9.1)	4 (0.6)	15.6	5.2-46.6	**<0.001**
Meconial	10 (5.1)	42 (6.7)	0.8	0.4-1.7	0.590
Translucent	169 (86)	584 (93)	1.0 (ref.)		

SD: Standard deviation; Ref.: reference category; In bold, significant *p*-values are highlighted.

In the multivariate analysis, the maternal variables predictors of AI <8 in the first minute of life were: mother's age >25 years (OR: 1.8; 95%CI: 1.1 – 2.8; $p = 0.020$) and absence of prenatal care (OR: 2.8; 95%CI: 1.5 – 5.5; $p = 0.010$). Additionally, predictive neonatal variables were: umbilical arterial pH (<7.0) (OR: 5.2; 95%CI: 4.0 – 8.8; $p < 0.001$), base excess (>-6.5) (OR: 4.1; 95%CI: 3.3 – 10.5; $p < 0.001$), fetal breech presentation (OR: 7.1; 95%CI: 4.2 – 13.1; $p < 0.001$), gestational age <33 weeks (OR: 15.1; 95%CI: 5.8 – 20.3; $p < 0.001$), fetal weight <2500g (OR: 21.5; 95%CI: 12.1 – 31.9; $p < 0.001$) and hemorrhagic amniotic fluid (OR: 13.8; 95%CI: 5.4 – 48.6; $p < 0.001$) (Table 3).

Table 3: Multivariate analysis to identify predictors associated with an AI outcome equal to zero in the first minute of life.

Variables	OR	CI95%	*p-values*
Mother variables			
Mother age (>25 years)	1.8	1.1 - 2.8	**0.020**
Maternal weight (<65 Kg)	1.1	0.5 - 1.9	0.297
Schooling (≤8 years)	1.9	0.4 - 2.7	0.157
Self-declared skin color (not white)	1.8	0.7 - 2.2	0.214
Hypertensive syndrome	1.2	0.8 - 5.5	0.312
Diabetic syndrome	0.5	0.7 - 5.3	0.302
Absence of prenatal	2.8	1.5 - 5.5	**0.010**
Parity (1)	1.1	0.7 - 1.88	0.267
Smoking	1.4	0.6 - 4.6	0.315
Alcohol consumption	2.5	0.4 - 5.1	0.247

Continues...

Table 3: Continuation.

Variables	OR	CI95%	*p-values*
Neonatal variables			
Umbilical arterial pH (≤7.0)	5.2	4.0 - 8.8	**<0.001**
Base excess (mmol/L) (>-6.5)	4.1	3.3 - 10.5	**<0.001**
Type of childbirth (cesarean)	0.8	0.3 - 1.2	0.191
Fetal presentation (pelvic)	7.1	4.2 - 13.1	**<0.001**
Gestacional age (<33 weeks)	15.1	5.8 - 20.3	**<0.001**
Fetal weight (<2.500g)	21.5	12.1 - 31.9	**<0.001**
Male sex	0.7	0.4 - 1.9	0.914
Amniotic fluid (Hemorrhagic)	13.8	5.4 - 48.6	**<0.001**

In bold, significant *p*-values are highlighted.

DISCUSSION

In the present study, neonatal mortality in the delivery room of NBs with zero AI in the first minute was 4.6% (n=10), and early neonatal mortality (<7 days) was 88% (n=193). It is known that early neonatal mortality, especially in the first six days of life, is associated with obstetric, maternal, childbirth, and prenatal care factors[15]. The AI in the fifth minute was also low, ranging from 0 to 5, which characterizes the significant fetal acidemia and difficulty in recovering these NBs[3,5,8]. The group of cases also had lower umbilical artery pH values and higher rates of base excess when blood gas was obtained from the umbilical artery. To better identify situations that compromise fetal health, umbilical artery pH has been widely adopted as a complement to AI[1,16-19]. Fetal acidemia has been suggested as having pH <7.0. It is known, however, that labor can induce a transient acidemia with a pH that can drop to 7.0 without harm to the fetus[8,19], and that some NBs with low AI may have normal pH values[17,19]. Pathological fetal acidemia can be defined by an umbilical artery pH of <7.0, which characterized the cases NBs in this study and signaled an increased risk of neurological damage and multiple organ dysfunctions[16,18,19]. The identified condition of acidosis explains the high rate of need for NB treatment in a neonatal intensive care environment. Previous studies cite that cases of low AI at birth, associated with markers of fetal acidemia are related to higher rates of neonatal morbidity and mortality[8,10,16,19].

The profile of pregnant women included in cases and controls was characterized by a mean maternal age of 26.8 ± 7.5 vs. 25.3 ± 6.9, mostly white, the average weight of 65.7 ± 15.6 vs. 66.2 ± 15.8 and identical parity, data without statistical significance to configure the homogeneous distribution of the studied sample. Haddad et al.(2001)[9] analyzed the obstetric history of conceptuses dead with successful resuscitation and associated them with black and multiparous patients, as well as the mean age and weight similar to those of the present study. It is important to emphasize that maternal and neonatal complications in low-risk pregnancies are likely to occur unexpectedly in primiparous patients (41.0%), with at least one complication, then in multiparous patients (19%) with at least one complication[19]. Primiparous women tend to have more complications during childbirth since labor/childbirth is generally longer and has a higher rate of dystocia than multiparous women[7].

Kassar et al. (2013)[14] when studying the determinants related to neonatal death with a focus on prenatal care and birth identified that schooling, maternal age, and family income were also not independent risk factors for early neonatal death[20]. Regarding the use of alcohol and tobacco, although the patients in cases had a higher percentage of users compared to controls, this percentage was small and the results were not statistically significant. The difficulty in knowing the real rate of these dependencies is well known.

The gender of the newborns was also not related to the outcome studied in this study. As for the obstetric and prenatal variables, it was evidenced that patients who did not undergo prenatal care are three times more likely to have birthed with zero AI in the 1st minute. Adequate prenatal care has been presented as one of the main protective factors against low birth weight, prematurity, intrauterine growth retardation, and, mainly, neonatal deaths[1,9,20]. In the study, birth weight was subdivided into those born weighing <2,500g and ≥2,500g, since high-risk newborns tend to weigh <2,500g[6,20]. In this study, 75% of the patients with AI zero weighed <2,500g (OR = 22.6; *p* <0.001). In the study by Haddad et al. (2001)[9] fetal weight was also lower in the group of apparently dead fetuses (*p* <0.001), as well as in the study by Madi et al. (2011)[1] that showed that lower gestational age was associated with a higher chance

of zero AI (p <0.001), as in the previously mentioned studies[1,9]. It is known, however, that AI may not be adequately evaluated in premature fetuses[2,11].

Regarding the mode of childbirth, in previous studies, c-section delivery was presented as a risk factor for complications and early neonatal outcomes[1,9,18]. In the study carried out at a public hospital in southern Brazil, the mode of childbirth was not statistically significant. In the case group, the percentage of pregnant women who developed hypertensive syndrome (n=31; 14%) was slightly higher than in controls (n=81; 12%).

The present study has some limitations: i) It is an observational study, and the findings must be taken into account with caution, given the degree of evidence of this type of design; ii) Sampling was by non-probabilistic convenience, which may have possibly included some sample collection bias, even although all epidemiological care was taken in this process; iii) It was not possible to access some clinical information such as length of stay, case-mix, medications used during hospitalization and outcomes such as seizures.

CONCLUSION

The AI zero was associated with the absence or inadequacy of prenatal care, breech fetal presentation, hemorrhagic amniotic fluid, prematurity, fetal weight <2,500g, and low AI up to the fifth minute of life. In addition, newborns were 10 times more likely to have neonatal death in the delivery room and had a greater need for treatment in a neonatal intensive care environment, as evidenced by lower pH values and higher values of base excess. Among the maternal variables, age and absence of prenatal care were predictors of the AI <8 in the first minute of birth.

Authorship

Jonas Wolf designed and implemented the study. Jonas Wolf performed the statistical analyses. Jonas Wolf wrote the first draft of the manuscript and contributed to the literature review and discussion of the results.

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Conflicts of interest

The authors declare no conflicts of interest.

Data availability statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

REFERENCES

1. Cnattingius S, Johansson S, Razaz N. Apgar Score and Risk of Neonatal Death among Preterm Infants. *N Engl J Med*. 2020;383(1):49-57. https://doi.org/10.1056/nejmoa1915075
2. Schlatter EF. Assessing the newborn infant's vitality by Apgar score. *Rev Esc Enferm USP*. 1981;15(3):267-73.
3. Iliodromiti S, Mackay DF, Smith GCS, Pell JP, Nelson SM. Apgar score and the risk of cause-specific infant mortality: a population-based cohort study. *Lancet*. 2014;384(9956):1749-55. https://doi.org/10.1016/s0140-6736(14)61135-1
4. Cnattingius S, Norman M, Granath F, Petersson G, Stephansson O, Frisell T. Apgar Score Components at 5 Minutes: Risks and Prediction of Neonatal Mortality. *Paediatr Perinat Epidemiol*. 2017;31(4):328-337. https://doi.org/10.1111/ppe.12360
5. Siddiqui A, Cuttini M, Wood R, Velebil P, Delnord M, Zile I, et al. Can the Apgar Score be Used for International Comparisons of Newborn Health? *Paediatr Perinat Epidemiol*. 2017;31(4):338-345. https://doi.org/10.1111/ppe.12368
6. Secretaria de Saúde do Estado do Paraná (BR). Caderno de Atenção à Saúde da Criança: recém-nascido de risco. Curitiba: Secretaria de Estado da Saúde; 2014.
7. Saraiva JP, Vogt SE, Rocha JS, Duarte ED, Simão DAS. Associação entre fatores maternos e neonatais e o Apgar em recém-nascidos de risco habitual. *Rev Rene*. 2018;19:e3179. https://doi.org/10.15253/2175-6783.2018193179
8. Kapaya H, Williams R, Elton G, Anumba D. Can Obstetric Risk Factors Predict Fetal Acidaemia at Birth? A Retrospective Case-Control Study. *J Pregnancy*. 2018;2018:2195965. https://doi.org/10.1155/2018/2195965
9. Haddad B, Mercer BM, Livingston JC, Sibai BM. Obstetric antecedents to apparent stillbirth (Apgar score zero at 1 minute only). *Obstet Gynecol*. 2001;97(6):961–4. https://doi.org/10.1016/s0029-7844(01)01352-7
10. Oliveira TG, Freire PV, Moreira FT, Moraes JSB, Arrelaro RC, Ricardi SRVA, et al. Apgar score and neonatal mortality in a hospital located in the southern area of São Paulo City, Brazil. *Einstein (Sao Paulo)*. 2012;10(1):22-8. https://doi.org/10.1590/S1679-45082012000100006
11. Catlin EA, Carpenter MW, Brann 4th BS, Mayfield SR, Shaul PW, Goldstein M, et al. The Apgar score revisited: influence of gestational age. *J Pediatr*. 1986;109(5):865-8. https://doi.org/10.1016/S0022-3476(86)80715-6
12. Oliveira LL, Gonçalves AC, Costa JSD, Bonilha ALL. Maternal and neonatal factors related to prematurity. *Rev Esc Enferm USP*. 2016;50(3):382–9. https://doi.org/10.1590/S0080-623420160000400002

13. Costa ALRR, Araujo Júnior E, Lima JWO, Costa FS. Fatores de risco materno associados à necessidade de terapia intensiva neonatal. *Rev Bras Ginecol Obstet*. 2014;36(1):29-34. https://doi.org/10.1590/S0100-72032014000100007

14. Kassar SB, Melo AMC, Coutinho SB, Lima MC, Lira PIC. Determinants of neonatal death with emphasis on health care during pregnancy, childbirth and reproductive history. *J Pediatr (Rio J)*. 2013;89(3):269-77. https://doi.org/10.1016/j.jped.2012.11.005

15. Westgate J, Garibaldi JM, Greene KR. Umbilical cord blood gas analysis at delivery: a time for quality data. *Br J Obstet Gynaecol*. 1994;101(12):1054-63. https://doi.org/10.1111/j.1471-0528.1994.tb13581.x

16. American Academy of Pediatrics, American College of Obstetricians and Gynecologists. The Apgar score. *Pediatrics*. 2006;117(4):1444-7.

17. De Zorzi PM, Madi JM, Rombaldi RL, Araújo, Zatti H, Madi SRC, et al. Fatores perinatais associados a recém-nascidos de termo com pH<7,1 na artéria umbilical e índice de Apgar <7,0 no 5º minuto. *Rev Bras Ginecol Obstet*. 2012;34(8):381-5. https://doi.org/10.1590/S0100-72032012000800007

18. Danilack VA, Nunes AP, Phipps MG. Unexpected complications of low-risk pregnancies in the United States. *Am J Obstet Gynecol*. 2015;212(6):809.e1-6. https://doi.org/10.1016/j.ajog.2015.03.038

19. Locatelli A, Incerti M, Ghidini A, Greco M, Villa E, Paterlini G. Factors associated with umbilical artery acidemia in term infants with low Apgar scores at 5 min. *Eur J Obstet Gynecol Reprod Biol*. 2008;139(2):146-50. https://doi.org/10.1016/j.ejogrb.2008.01.003

20. Gardner MO, Goldenberg RL, Gaudier FL, Dubard MB, Nelson KG, Hauth JC. Predicting low Apgar scores of infants weighing less than 1000 grams: the effect of corticosteroids. *Obstet Gynecol*. 1995;85(2):170-4. https://doi.org/10.1016/0029-7844(94)00372-K

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Original Article

ENTEROVIRUS D68: EPIDEMIOLOGY OF A VIRUS THAT CAUSES PEDIATRIC INFECTIOUS DISEASE

Jonas Wolf[1]

ABSTRACT

Introduction: Enterovirus D68 (EV-D68) is a respiratory virus that primarily affects children and has been associated with sporadic outbreaks of respiratory tract disease worldwide. This study aimed to carry out a literature review considering several aspects involving EV-D68 infection: epidemiology, molecular evolution, prevention, diagnosis, and treatment of this infection.

Methods: Data from 976 whole genome sequences (WGS) of EV-D68 collected between September 1977 to September 2022 showed substitution rates of 4.06E10-3 nucleotides per site per year (s/s/y). Phylogenetic tree of EV-D68 by clades (A1, A2, B, B1, B2, B3, and C) was performed.

Results: Literature reports that EV-D68 was discovered in 1962 after being isolated from respiratory specimens of children with pneumonia in the USA. However, in the recent molecular analysis from Nextstrain data, we observed that the time to the most recent common ancestor (tMRCA) of A1 was 2005-04-17 in the USA, A2 was 2003-12-23 in China, B was 2003-07-06 in China, B1 was 2010-03-21 in Vietnam, B2 was 2006-11-25 in Vietnam, B3 was 2011-01-15 in China, and C was 2000-06-27 in the USA. The immune response to EV-D68 involves both innate and adaptive immunity, with the production of neutralizing antibodies and activation of T cells playing crucial roles. Prevention strategies for EV-D68 include practicing good hand hygiene, respiratory etiquette, and avoiding close contact with infected individuals.

Conclusion: EV-D68 was originated in Canada in 1995 and spread to Europe, Asia, Oceania, Latin America, and Africa.

Keywords: *Enterovirus D68; Respiratory Tract Diseases; Molecular Evolution; Epidemiology.*

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INTRODUCTION

Enteroviruses are a large group of viruses that belong to the Picornaviridae family. They are single-stranded RNA viruses and are known to cause a wide range of human illnesses. Enteroviruses are classified into several species, including Enterovirus A, Enterovirus B, Enterovirus C, and Enterovirus D[1-5].

Enteroviruses are transmitted primarily through the fecal-oral route, although they can also be spread through respiratory droplets and direct contact with contaminated surfaces. They can cause a variety of illnesses, ranging from mild respiratory infections, gastrointestinal disorders, and hand, foot, and mouth disease to more severe conditions such as meningitis, encephalitis, myocarditis, and acute flaccid myelitis[1,3-5].

Among the different species of enteroviruses, Enterovirus D68 (EV-D68) has gained attention in recent years due to its association with respiratory illness outbreaks, particularly in children. EV-D68 typically causes symptoms similar to the common cold, including coughing, sneezing, runny nose, and fever. However, in some cases, particularly in individuals with pre-existing respiratory conditions, EV-D68 can lead to severe respiratory illness, requiring hospitalization[5-9].

EV-D68 is a type of virus that belongs to the family Picornaviridae, specifically the Enterovirus genus. It was first identified in California, United

States, in 1962. EV-D68 primarily affects children and causes respiratory illness, ranging from mild cold-like symptoms to more severe respiratory complications.[6,10] The virus is spread through respiratory secretions, such as saliva, nasal mucus, or sputum, when an infected person coughs, sneezes or touches contaminated surfaces. It can also spread through close contact with an infected individual. EV-D68 tends to be more common during the late summer and fall seasons[6,10,11].

For several decades following its initial discovery, EV-D68 remained relatively uncommon and caused only sporadic outbreaks. However, in recent years, there have been notable increases in the number of EV-D68 cases reported worldwide. The largest outbreak to date occurred in 2014 in the United States, where multiple states reported a significant number of cases, particularly among children with asthma or a history of adventitious sounds[5,6,10,11].

During the 2014 outbreak, EV-D68 gained widespread attention due to the severity of respiratory illness it caused in some individuals. Many children with EV-D68 infection experienced difficulty breathing, wheezing, and respiratory distress, leading to hospitalization in some cases. This raised concerns about the virus's potential to cause severe respiratory complications, especially in individuals with pre-existing respiratory conditions[5-7,10].

Since 2014, EV-D68 continues to circulate, and sporadic outbreaks have been reported in various countries. However, the frequency and intensity of outbreaks can vary from year to year. It is important to note that while EV-D68 can cause severe respiratory illness, most individuals who contract the virus experience mild symptoms and recover without complications[5,7,10].

Prevention and control measures for EV-D68 are similar to those recommended for other respiratory viruses. These include regular handwashing with soap and water, covering the mouth and nose when coughing or sneezing, avoiding close contact with sick individuals, and disinfecting frequently-touched surfaces. Individuals with asthma or other respiratory conditions should ensure their condition is well-managed and follow their healthcare provider's guidance[5,6,10,11].

The exact mortality rate associated with EV-D68 is challenging to determine as it varies depending on factors such as population demographics, healthcare access, and the availability of supportive care. Additionally, not all cases of EV-D68 infection are reported, which can affect the accuracy of mortality data. In a meta-analysis, was identified the highest EV-D68 prevalences were in hospital outbreaks, in developed countries, in children under 5, and in patients with acute flaccid myelitis and asthma-related diseases. Sporadic deaths linked can occur associated with severe respiratory EV-D68 infections. EV-D68 shows a low prevalence of current as opposed to the existence of EV-D68 antibodies in almost all children. Therefore, highlight the need to implement and/or strengthen continuous surveillance of EV-D68 infections in hospitals and in the community for the anticipation of the response to future epidemics[5,12].

This study aimed to carry out a literature review considering several aspects involving EV-D68 infection: epidemiology, molecular evolution, prevention, diagnosis, and treatment of this infection.

METHODS

The keywords "Enterovirus D68 global distribution", "Enterovirus D68 prevalence", "Enterovirus D68 biology", "Enterovirus D68 molecular evolution", "Enterovirus D68 phylodynamics", "Enterovirus D68 phylogeography", "Enterovirus D68 immunology", "Enterovirus D68 diagnosis", "Enterovirus D68 prevention", "Enterovirus D68 therapeutics", were used with Boolean combinations. The literature search and relevance evaluation were conducted with the databases PubMed, Web of Science, and Google Scholar. Articles found were considered potential reference sources. Searches were performed up to early June 2023.

RESULTS AND DISCUSSION

Enterovirus D68 epidemiology

EV-D68 has been reported in many countries, including the United States, Canada, European countries, and parts of Asia. The frequency and intensity of outbreaks may vary from year to year and between regions[12-25]. EV-D68 infections primarily occur during late summer and fall, typically peaking between August and October in the Northern Hemisphere. However, sporadic cases can be reported throughout the year.[19,26] EV-D68 infections predominantly affect children, particularly those between the ages of 4 and 12 years. This age group is more susceptible to the virus, possibly due to lower pre-existing immunity[19,26,27].

EV-D68 is known for causing respiratory illness, ranging from mild respiratory symptoms resembling the common cold to severe respiratory complications. It can lead to wheezing, difficulty breathing, and in some cases, hospitalization. Children with asthma or a history of wheezing are at higher risk of developing severe symptoms[19,26].

EV-D68 outbreaks have been associated with clusters of cases in various settings, including schools, daycare centers, and communities. These outbreaks often draw attention due to an increased number of severe respiratory illnesses reported within a specific time frame.[19,28] Enhanced surveillance systems are in place in many countries to monitor EV-D68 activity. Public health agencies and laboratories actively monitor

respiratory illness patterns to detect and investigate outbreaks, identify circulating strains, and track changes in the virus over time[19]. EV-D68 is part of a larger group of enteroviruses that show seasonal patterns. Other enteroviruses, such as enterovirus A71, coxsackievirus, and echovirus, may circulate during the same season and cause similar respiratory symptoms[19,28]. This virus exhibits genetic diversity, with multiple distinct clades identified worldwide. Molecular characterization helps in understanding the virus's evolution, tracking its spread, and identifying potential changes in virulence or transmissibility[19,27,29].

Molecular evolution of Enterovirus D68

EV-D68 is a positive-sense, single-stranded RNA virus belonging to the Enterovirus genus within the Picornaviridae family. Its genome is approximately 7.4 kilobases in length and contains a single open reading frame (ORF) flanked by untranslated regions (UTRs) at the 5' and 3' ends[6,7,19,27,30].

EV-D68 exhibits genetic diversity, and different clades of the virus have been identified based on genetic variations in the VP1 gene, which encodes the major capsid protein. Multiple genotypes, such as clade A, B1, B2, and C, have been described, with varying distribution and prevalence in different regions and over time[27,29,30].

Phylogenetic analyses have revealed that EV-D68 is closely related to other enteroviruses, including human rhinoviruses (HRVs) and other enterovirus species, such as enterovirus A and enterovirus C. This suggests common ancestry and evolutionary relationships with these viruses[6,7,29]. Recombination, a process where genetic material is exchanged between different viral strains, has been reported in EV-D68. Recombination events can lead to the emergence of new clades and contribute to the genetic diversity of the virus. The precise impact of recombination on EV-D68 evolution and its implications for virulence or transmissibility are still being studied[7,27,30,31].

The evolutionary dynamics of EV-D68 are complex and influenced by various factors, including immune pressure, viral fitness, host range, and population immunity. Evolutionary changes can occur through genetic mutations, recombination events, and selection pressures, leading to the emergence of new strains or variants over time[6,9,29-32]. Molecular analysis has shown that different EV-D68 clades have circulated globally, with some clades being more prevalent in certain regions or during specific outbreak periods. Genomic surveillance helps track the spread of different clades and understand their geographic distribution[29,33].

The relationship between EV-D68 genetic variations and virulence or pathogenicity is an active area of research. Some studies have suggested that certain genotypes or specific amino acid changes in viral proteins, such as the VP1 capsid protein, may be associated with increased disease severity or the ability to cause respiratory complications. However, more research is needed to fully understand the molecular determinants of EV-D68 pathogenicity[6,7,19,27,30].

Phylodynamic and phylogeographic analysis of Enterovirus D68

Data from nextstrain (https://nextstrain.org/enterovirus/d68/genome) with 976 whole genome sequences (WGS) of EV-D68 collected between September 1977 to September 2022 showed substitution rates of 4.06E10-3 (Highest Posterior Density [HPD 95%]: 3.88E10-3 to 4.35E10-3) nucleotides per site per year (s/s/y) (Figure 1).

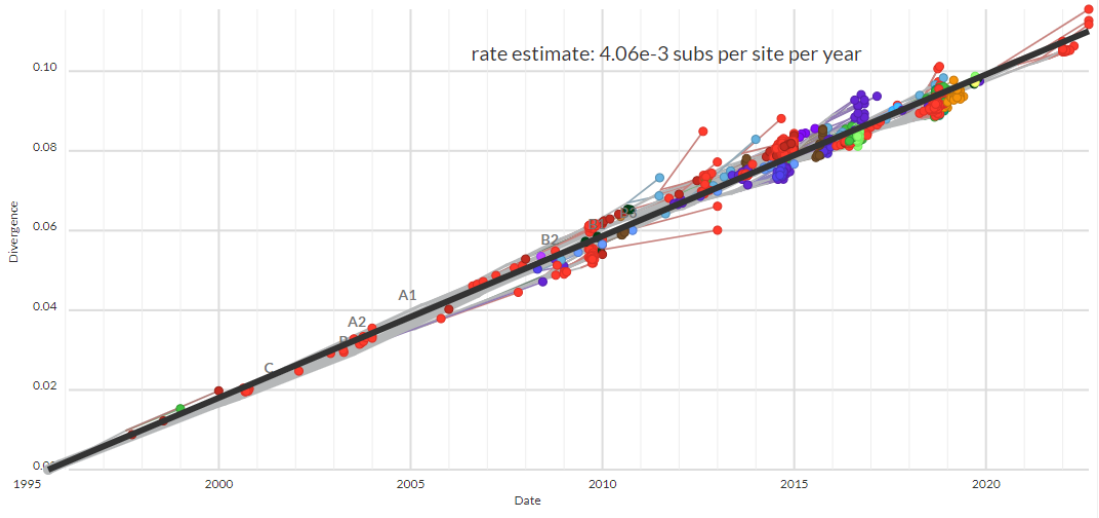

Figure 1: Root-to-tip regression of 976 complete genomes of enterovirus D68 (sequences collected between September 1977 and September 2022) by countries obtained from GISAID.

Figure 2 shows the phylogenetic tree of EV-D68 WGS in the world by clades (A1, A2, B, B1, B2, B3, and C). Analysis of recent evolution of EV-D68 shown that the time to the most recent common ancestor (tMRCA) of A1 was 2005-04-17 (Highest Posterior Density [HPD95%]: 2005-01-09; 2005-09-01) in the USA, A2 was 2003-12-23 (HPD95%: 2003-07-14; 2004-06-06) in China, B was 2003-07-06 (HPD95%: 2003-01-21; 2003-09-28) in China, B1 was 2010-03-21 (HPD95%: 2009-12-20; 2010-05-19) in Vietnam, B2 was 2006-11-25 (HPD95%: 2006-09-07; 2007-03-19) in Vietnam, B3 was 2011-01-15 (HPD95%: 2010-09-07; 2011-03-30) in China, and C was 2000-06-27 (HPD95%: 2000-05-21; 2000-07-24) in the USA.

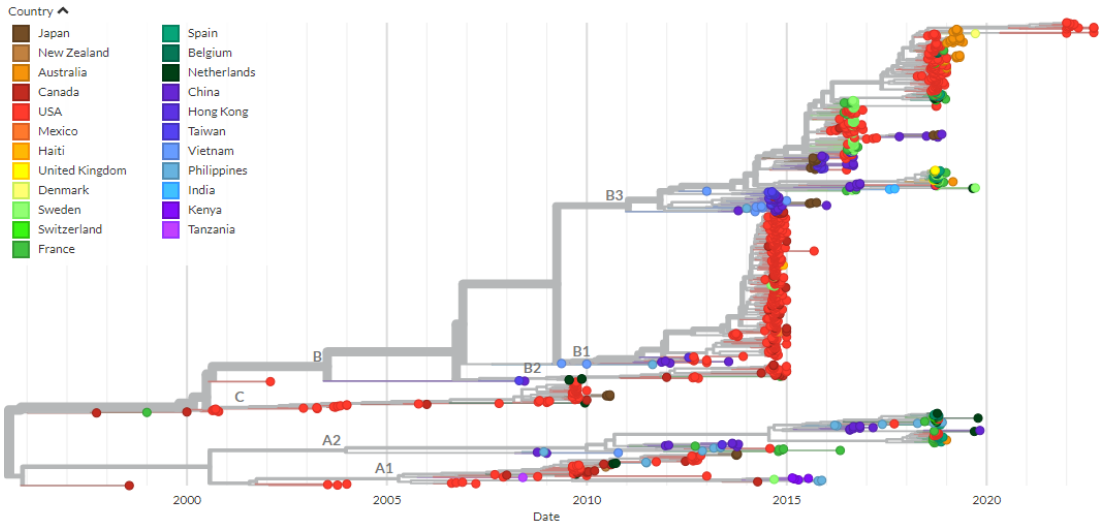

Figure 2: Time-scaled maximum clade credibility tree from the evolutionary reconstruction by Bayesian analysis of enterovirus D68 (976 complete genome sequences collected between September 1977 and September 2022) by countries obtained from GISAID.

The geographic distribution of EV-D68 clades that circulated in the world is shown in Figures 3 and 4. The molecular origin of the EV-D68 was in Canada in 1995 (Bayes Factor [BF]=38), later it was disseminated in France in 1997 (BF=23), the USA in 1999 (BF=30), Taiwan in 2008 (BF=25), Tanzania in 2008 (BF=15), China in 2008 (BF=31), Philippines in 2008 (BF=10), Vietnam in 2009 (BF=15), the Netherlands in 2009 (BF=11), New Zealand in 2010 (BF=10), Japan in 2010 (BF=12), Hong Kong in 2012 (BF=17), Mexico in 2014 (BF=12), Kenya in 2015 (BF=8), Sweden in 2016 (BF=12), India in 2017 (BF=10), Switzerland in 2018 (BF=9), Spain in 2018 (BF=10), Belgium in 2018 (BF=8), Australia in 2018 (BF=9), and Denmark in 2019 (BF=10). Recently, in 2022 this virus circulated in the USA.

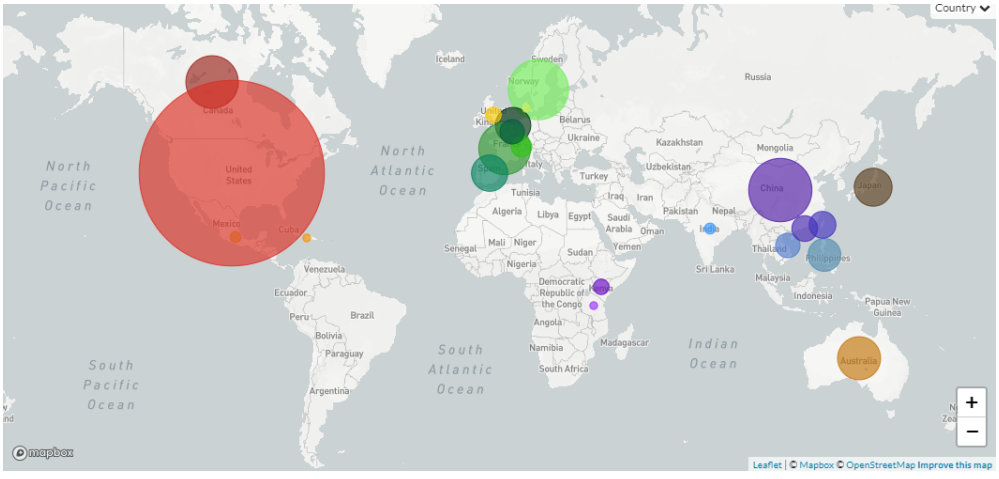

Figure 3: Enterovirus D68 dissemination in worldwide.

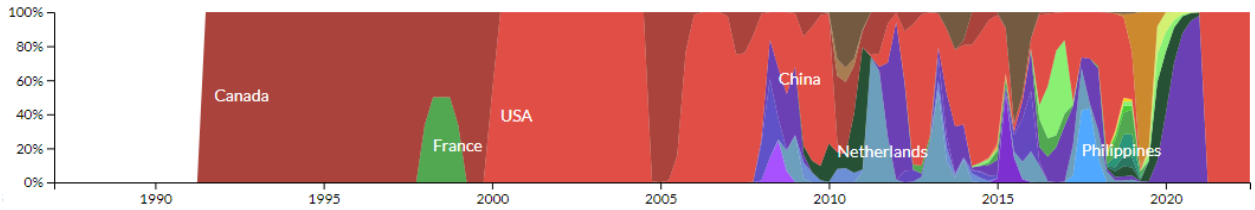

Figure 4: Frequencies of enterovirus D68 colored by country.

Enterovirus D68 immunology

Upon infection with EV-D68, the innate immune system is activated as the first line of defense. Innate immune cells, such as macrophages and dendritic cells, recognize viral components through pattern recognition receptors (PRRs) and initiate a cascade of immune responses. This includes the release of cytokines and chemokines that recruit and activate other immune cells[6,34-36].

The adaptive immune response plays a crucial role in clearing EV-D68 infection and providing long-term immunity. T lymphocytes, specifically CD4+ and CD8+ T cells, recognize viral antigens presented by infected cells. CD4+ T cells help coordinate the immune response by activating other immune cells, while CD8+ T cells directly target and kill infected cells[6,34-36].

B lymphocytes produce antibodies in response to EV-D68 infection. Neutralizing antibodies, which can bind to the virus and prevent its entry into host cells, are particularly important for limiting viral spread. The production of specific antibodies against viral proteins, such as the capsid protein VP1, contributes to the clearance of the virus[35,36].

Following EV-D68 infection, the immune system develops memory cells that provide long-term protection against reinfection. Memory B cells and memory T cells retain information about the virus, allowing for a faster and more effective immune response upon subsequent encounters with EV-D68[6,34-37].

Host Factors: Individual variations in the immune response can influence susceptibility to EV-D68 and the severity of the disease. Factors such as age, pre-existing immunity, and underlying health conditions, including respiratory disorders like asthma, can impact the immune response and contribute to differences in disease outcomes[6,10,34-38].

In some cases, EV-D68 infection has been associated with immune-mediated complications. It has been hypothesized that an overly robust or dysregulated immune response, including excessive production of pro-inflammatory cytokines, may contribute to respiratory symptoms and severe disease manifestations in susceptible individuals.[6,34-36,38] Efforts are underway to develop vaccines against EV-D68. Vaccine candidates aim to stimulate both antibody and T-cell responses to provide protective immunity. However, vaccine development for EV-D68 is challenging due to the genetic diversity of the virus and the need to target multiple genotypes.

Understanding the immunology of EV-D68 is essential for developing effective preventive measures, treatment options, and vaccines. Ongoing research continues to shed light on the immune response to EV-D68 infection and how it can be harnessed to mitigate the impact of the virus[6,10,34-36,38].

Enterovirus D68 prevention

Preventing the spread of EV-D68 involves implementing a combination of personal hygiene practices and public health measures. Here are some key prevention strategies:

Hand Hygiene: Regularly washing hands with soap and water for at least 20 seconds, especially before eating, after using the restroom, and after coughing or sneezing. If soap and water are not available, use an alcohol-based hand sanitizer that contains at least 60% alcohol[1-5].

Respiratory Etiquette: Cover mouth and nose with a tissue or your elbow when coughing or sneezing. Dispose of used tissues properly and immediately wash washing hands afterward. If a tissue is not available, cough or sneeze into elbow rather than your hands[4,5].

Avoid Close Contact: Limit close contact with individuals who are sick, particularly those displaying respiratory symptoms. Stay at least 6 feet away from people who are coughing, sneezing, or showing signs of illness[5].

Clean and Disinfect: Regularly clean and disinfect frequently-touched surfaces, such as doorknobs, light switches, countertops, and electronic devices. Use EPA-approved disinfectants that are effective against viruses[5].

Stay Home When Sick: If child are experiencing symptoms of respiratory illness, such as coughing, sneezing, or fever, it is important to stay home from work, school, or public places to prevent the spread of EV-D68 and other viruses[1-5,39].

Maintain Respiratory Health: Take steps to maintain good respiratory health, especially if you or your child have asthma or other respiratory conditions. Follow your healthcare provider's instructions for managing your condition and ensure medications are taken as prescribed[5].

Stay Informed: Stay updated with information and guidelines provided by local health authorities, such as the Centers for Disease Control and Prevention (CDC) or the World Health Organization (WHO). They can guide prevention strategies and any specific recommendations during outbreaks or high-risk periods[5].

Enterovirus D68 diagnosis

A healthcare provider will assess the patient's medical history, symptoms, and physical examination findings. EV-D68 primarily causes respiratory illness, so symptoms like coughing, sneezing, runny nose, wheezing, and difficulty breathing may raise suspicion of EV-D68 infection. However, these symptoms can also be caused by other respiratory viruses, so laboratory confirmation is essential[10,40-43].

The preferred method for EV-D68 diagnosis is molecular detection through reverse transcription-polymerase chain reaction (RT-PCR) assays. These tests detect the presence of EV-D68 RNA in respiratory specimens, such as nasal or throat swabs or nasopharyngeal aspirates. RT-PCR provides rapid and specific identification of the virus[8]. Serological testing, specifically testing for the presence of EV-D68-specific antibodies in blood samples, can be performed to determine past exposure or recent infection. However, serological testing is less commonly used for acute diagnosis due to the need for paired acute and convalescent samples[27].

Knowledge of EV-D68 activity in the community or region can assist in the diagnosis. If EV-D68 outbreaks are occurring or there is a high prevalence of cases in the area, it increases the likelihood of EV-D68 infection in symptomatic individuals[10,40-44]. It is important to consult with a healthcare professional for accurate diagnosis and guidance on appropriate testing. EV-D68 shares symptoms with other respiratory viruses, and laboratory confirmation is necessary to distinguish it from other similar illnesses. Additionally, healthcare providers may consider testing for other respiratory pathogens to rule out co-infections or alternative diagnoses[10,43,45].

Prompt diagnosis of EV-D68 is crucial for appropriate patient management, implementation of infection control measures, and surveillance purposes. Healthcare providers should follow local guidelines and consult with public health authorities for specific recommendations on EV-D68 diagnosis and reporting[10,40,43].

Enterovirus D68 treatment

Currently, there is no specific antiviral treatment or vaccine available for EV-D68. Treatment for EV-D68 is primarily supportive and focuses on relieving symptoms and managing complications. Here are some key aspects of EV-D68 treatment:

Symptom Relief: Over-the-counter pain relievers and fever reducers, such as acetaminophen or ibuprofen, can help alleviate fever, muscle aches, and discomfort. It is important to follow the recommended dosage instructions and consult a healthcare provider if there are any concerns or if the symptoms worsen[10,45,46].

Respiratory Support: Individuals with severe respiratory symptoms, such as wheezing or difficulty breathing, may require medical intervention. In such cases, healthcare providers may administer bronchodilators to help open the airways or provide supplemental oxygen therapy to support breathing[10,46].

Fluids and Rest: It is important to stay well-hydrated by drinking plenty of fluids, especially if there is fever or respiratory symptoms. Getting adequate rest is also crucial for the body's recovery and immune response.

Individuals with pre-existing asthma or other respiratory conditions should ensure that their condition is well-managed. Following the prescribed asthma management plan, using prescribed inhalers or medications as directed, and monitoring symptoms are important to prevent exacerbations and complications. In severe cases, particularly when respiratory distress is present, hospitalization may be necessary. Hospital-based care can provide intensive monitoring, oxygen therapy, and other interventions to support the patient's respiratory function and overall well-being[10,45,46].

CONCLUSION

EV-D68 is a respiratory virus that primarily affects children and can cause a range of respiratory symptoms, from mild cold-like symptoms to severe respiratory illness. The epidemiology of EV-D68 is characterized by sporadic outbreaks, often occurring during late summer and fall. Enhanced surveillance helps monitor it is activity and track circulating strains.

Literature reports that EV-D68 was discovered in 1962 after being isolated from respiratory specimens of children with pneumonia in the USA. The molecular evolution of EV-D68 involves genetic diversity, with multiple clades identified worldwide. EV-D68 spread in North America to Europe, Asia, Oceania, Latin America, and Africa. Recently, in 2022 this virus circulated mainly in the USA.

The immune response to EV-D68 involves both innate and adaptive immunity, including the production of neutralizing antibodies and the activation of T cells. Individual factors, such as age and underlying health conditions, can influence the immune response and disease outcomes.

Preventing the spread of EV-D68 involves practicing good hand hygiene, respiratory etiquette, and avoiding close contact with sick individuals. Regular cleaning and disinfection of surfaces are important preventive measures. Staying informed and following guidelines from health authorities is crucial in preventing and managing EV-D68 infections. Currently, there is no specific antiviral treatment or vaccine available for EV-D68. Treatment focuses on supportive care, including symptom relief, respiratory support if necessary, adequate hydration, rest, and management of underlying health conditions.

Also, it is important to note that the molecular evolution of EV-D68 is an ongoing field of study, and new findings may emerge as research progresses. Continued surveillance and genetic analysis are essential for monitoring the virus's evolutionary changes and informing public health interventions.

Authorship

Jonas Wolf designed the study. Jonas Wolf wrote the first draft of the manuscript and contributed to the literature review and discussion of the results.

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Conflicts of interest

The author declare no conflicts of interest.

Data availability statement

Data sharing is not applicable to this article as no new data were created or analyzed in this study

REFERENCES

1. Tapparel C, Siegrist F, Petty TJ, Kaiser L. Picornavirus and enterovirus diversity with associated human diseases. *Infect Genet Evol*. 2013;14:282-93. https://doi.org/10.1016/j.meegid.2012.10.016

2. Jubelt B, Lipton HL. Enterovirus/picornavirus infections. *Handb Clin Neurol*. 2014;123:379-416. https://doi.org/10.1016/B978-0-444-53488-0.00018-3

3. Crom SC, Rossen JWA, van Furth AM, Obihara CC. Enterovirus and parechovirus infection in children: a brief overview. *Eur J Pediatr*. 2016;175(8):1023-9. https://doi.org/10.1007/s00431-016-2725-7

4. Pham NTK, Thongprachum A, Trinh QD, Okitsu S, Komine-Aizawa S, Shimizu H, et al. Detection and genetic characterization of enterovirus strains circulating among children with acute gastroenteritis in Japan during 2014-2016. *Infect Genet Evol*. 2018;61:16-9. https://doi.org/10.1016/j.meegid.2018.03.009

5. Non-polio enteroviruses 2020 [Internet]. Washington, DC: Centers for Disease Control and Prevention. c2020 [cited 2023 Jun 30]. Available from: https://www.cdc.gov/non-polio-enterovirus/index.html

6. Elrick MJ, Pekosz A, Duggal P. Enterovirus D68 molecular and cellular biology and pathogenesis. *J Biol Chem*. 2021;296:100317. https://doi.org/10.1016/j.jbc.2021.100317

7. Ayudhya SSN, Laksono BM, Riel D. The pathogenesis and virulence of enterovirus-D68 infection. *Virulence*. 2021;12(1):2060-72. https://doi.org/10.1080/21505594.2021.1960106

8. Andrés C, Vila J, Creus-Costa A, Piñana M, González-Sánchez A, Esperalba J, et al. Enterovirus D68 in hospitalized children, Barcelona, Spain, 2014-2021. *Emerg Infect Dis*. 2022;28(7):1327-31. https://doi.org/10.3201/eid2807.220264

9. Fischer TK, Simmonds P, Harvala H. The importance of enterovirus surveillance in a post-polio world. *Lancet Infect Dis*. 2022;22(1):e35-e40. https://doi.org/10.1016/S1473-3099(20)30852-5

10. Sun J, Hu XY, Yu XF. Current understanding of human enterovirus D68. *Viruses*. 2019;11(6):490. https://doi.org/10.3390/v11060490

11. Hu Y, Musharrafieh R, Zheng M, Wang J. Enterovirus D68 antivirals: past, present, and future. *ACS Infect Dis*. 2020;6(7):1572-86. https://doi.org/10.1021/acsinfecdis.0c00120

12. Fall A, Kenmoe S, Ebogo-Belobo JT, Mbaga DS, Bowo-Ngandji A, Foe-Essomba JR, et al. Global prevalence and case fatality rate of enterovirus D68 infections, a systematic review and meta-analysis. *PLoS Negl Trop Dis*. 2022;16(2):e0010073. https://doi.org/10.1371/journal.pntd.0010073

13. Ikuse T, Aizawa Y, Yamanaka T, Habuka R, Watanabe K, Otsuka T, et al. Outbreak of enterovirus D68 among children in Japan – worldwide circulation of enterovirus D68 clade B3 in 2018. Pediatr Infect Dis J. 2021;40(1):6-10. https://doi.org/10.1097/INF.0000000000002889

14. Pellegrinelli L, Giardina F, Lunghi G, Renteria SCU, Greco L, Fratini A, et al. Emergence of divergent enterovirus (EV) D68 sub-clade D1 strains, northern Italy, September to October 2018. *Euro Surveill*. 2018;24(7):1900090. https://doi.org/10.2807/1560-7917.ES.2018.24.7.1900090

15. Liu Y, Gong C, Luo M, Zhang T, Li M, Shen L, et al. Seroepidemiology of enterovirus D68 in a healthy population in Beijing, China, between 2012 and 2017: a retrospective study. *J Med Virol*. 2021;93(6):3524-31. https://doi.org/10.1002/jmv.26132

16. Park SW, Pons-Salort M, Messacar K, Cook C, Meyers L, Farrar J, et al. Epidemiological dynamics of enterovirus D68 in the United States and implications for acute flaccid myelitis. *Sci Transl Med*. 2021;13(584):eabd2400. https://doi.org/10.1126/scitranslmed.abd2400

17. Shah MM, Perez A, Lively JY, Avadhanula V, Boom JA, Chappell J, et al. Enterovirus D68-associated acute respiratory illness – new vaccine surveillance network, United States, July-November 2018-2020. *MMWR Morb Mortal Wkly Rep*. 2021;70(47):1623-8. https://doi.org/10.15585/mmwr.mm7047a1

18. Chan YF, Sam IC, Nayan E, Tan XH, Yogarajah T. Seroepidemiology of enterovirus D68 infection in Kuala Lumpur, Malaysia between 2013 and 2015. *J Med Virol*. 2022;94(6):2607-12. https://doi.org/10.1002/jmv.27381

19. Hodcroft EB, Dyrdak R, Andrés C, Egli A, Reist J, Artola DGM, et al. Evolution, geographic spreading, and demographic distribution of enterovirus D68. *PLoS Pathog*. 2022;18(5):e1010515. https://doi.org/10.1371/journal.ppat.1010515

20. Larsson SB, Vracar D, Karlsson M, Ringlander J, Norder H. Epidemiology and clinical manifestations of different enterovirus and rhinovirus types show that EV-D68 may still have an impact on severity of respiratory infections. *J Med Virol*. 2022;94(8):3829-39. https://doi.org/10.1002/jmv.27767

21. Fall A, Gallagher N, Morris CP, Norton JM, Pekosz A, Klein E, et al. Circulation of enterovirus D68 during period of increased influenza-like illness, Maryland, USA, 2021. *Emerg Infect Dis*. 2022; 28(7):1525-27. https://doi.org/10.3201/eid2807.212603

22. Peltola V, Österback R, Waris M, Ivaska L, Tähtinen PA, Laine M, et al. Enterovirus D68 outbreak in children, Finland, August-September 2022. *Emerg Infect Dis*. 2023;29(6):1258-61. https://doi.org/10.3201/eid2906.221795

23. Stelzer-Braid S, Yeang M, Britton PN, Kim KW, Varadhan H, Andrews PI, et al. Circulation of enterovirus D68 (EV-D68) causing respiratory illness in New South Wales, Australia, between August 2018 and November 2019. *Pathology*. 2022;54(6):784-9. https://doi.org/10.1016/j.pathol.2022.03.007

24. Tedcastle A, Wilton T, Pegg E, Klapsa D, Bujaki E, Mate R, et al. Detection of enterovirus D68 in wastewater samples from the UK between July and November 2021. *Viruses*. 2022;14(1):143. https://doi.org/10.3390/v14010143

25. Pons-Salort M, Lambert B, Kamau E, Pebody R, Harvala H, Simmonds P, et al. Changes in transmission of enterovirus D68 (EV-D68) in England inferred from seroprevalence data. *eLife*. 2023;12:e76609. https://doi.org/10.7554/eLife.76609

26. Benschop KSM, Albert J, Anton A, Andrés C, Aranzamendi M, Armannsdóttir B, et al. Re-emergence of enterovirus D68 in Europe after easing the COVID-19 lockdown, September 2021. *Euro Surveill*. 2021;26(45):2100998. https://doi.org/10.2807/1560-7917.ES.2021.26.45.2100998

27. Ebada MA, Fayed N, Alkanj S, Allah AW. Enterovirus D-68 molecular virology, epidemiology, and treatment: an update and way forward. *Infect Disord Drug Targets*. 2021;21(3):320-7. https://doi.org/10.2174/1871526520666200715101230

28. Fall A, Han L, Abdullah O, Norton JM, Eldesouki RE, Forman M, et al. An increase in enterovirus D68 circulation and viral evolution during a period of increased influenza like illness, The Johns Hopkins Health System, USA, 2022. *J Clin Virol*. 2023;160:105379. https://doi.org/10.1016/j.jcv.2023.105379

29. Fang Y, Chen Q, Wang H, Wang L, Rong H, Liao Q, et al. The role of conformational epitopes in the evolutionary divergence of enterovirus D68 clades: a bioinformatics-based study. *Infect Genet Evol*. 2021;93:104992. https://doi.org/10.1016/j.meegid.2021.104992

30. Tan Y, Hassan F, Schuster JE, Simenauer A, Selvarangan R, Halpin RA, et al. Molecular evolution and intraclade recombination of enterovirus D68 during the 2014 outbreak in the United States. *J Virol*. 2016;90(4):1997-2007. https://doi.org/10.1128/JVI.02418-15

31. Gong YN, Yang SL, Shih SR, Huang YC, Chang PY, Huang CG, et al. Molecular evolution and the global reemergence of enterovirus D68 by genome-wide analysis. *Medicine (Baltimore)*. 2016;95(31):e4416. https://doi.org/10.1097/MD.0000000000004416

32. Wollants E, Beller L, Beuselinck K, Bloemen M, Lagrou K, Reynders M, et al. A decade of enterovirus genetic diversity in Belgium. *J Clin Virol*. 2019;121:104205. https://doi.org/10.1016/j.jcv.2019.104205

33. Tang SH, Yuan Y, Xie ZH, Chen MJ, Fan XD, Guo YH, et al. Enterovirus D68 in hospitalized children with respiratory symptoms in Guangdong from 2014 to 2018: molecular epidemiology and clinical characteristics. *J Clin Virol*. 2021;141:104880. https://doi.org/10.1016/j.jcv.2021.104880

34. Huang W, Wang G, Zhuge J, Nolan SM, Dimitrova N, Fallon JT. Whole-genome sequence analysis reveals the enterovirus D68 isolates during the United States 2014 outbreak mainly belong to a novel clade. *Sci Rep*. 2015;5:15223. https://doi.org/10.1038/srep15223

35. Huan C, Qu X, Li Z. Host restrictive factors are the emerging storm troopers against enterovirus: a mini-review. *Front Immunol*. 2022;13:910780. https://doi.org/10.3389/fimmu.2022.910780

36. Nguyen-Tran H, Park SW, Messacar K, Dominguez SR, Vogt MR, Permar S, et al. Enterovirus D68: a test case for the use of immunological surveillance to develop tools to mitigate the pandemic potential of emerging pathogens. *Lancet Microbe*. 2022;3(2):e83-e85. https://doi.org/10.1016/S2666-5247(21)00312-8

37. Harrison CJ, Weldon WC, Pahud BA, Jackson MA, Oberste MS, Selvarangan R. Neutralizing antibody against enterovirus D68 in children and adults before 2014 outbreak, Kansas City, Missouri, USA. *Emerg Infect Dis*. 2019;25(3):585-8. https://doi.org/10.3201/eid2503.180960

38. Wen X, Sun D, Guo J, Elgner F, Wang M, Hildt E, et al. Multifunctionality of structural proteins in the enterovirus life cycle. *Future Microbiol*. 2019;14(13):1147-1157. https://doi.org/10.2217/fmb-2019-0127

39. Moss RB. Enterovirus 68 infection – association with asthma. *J Allergy Clin Immunol Pract*. 2016;4(2):226-8. https://doi.org/10.1016/j.jaip.2015.12.013

40. Esposito S, Bosis S, Niesters H, Principi N. Enterovirus D68 infection. *Viruses*. 2015;7(11):6043-50. https://doi.org/10.3390/v7112925

41. Foster CB, Friedman N, Carl J, Piedimonte G. Enterovirus D68: a clinically important respiratory enterovirus. *Cleve Clin J Med*. 2015;82(1):26-31. https://doi.org/10.3949/ccjm.82a.14166

42. Xiang Z, Xie Z, Liu L, Ren L, Xiao Y, Paranhos-Baccalà G, et al. Genetic divergence of enterovirus D68 in China and the United States. *Sci Rep*. 2016;6:27800. https://doi.org/10.1038/srep27800

43. Harvala H, Broberg E, Benschop K, Berginc N, Ladhani S, Susi P, et al. Recommendations for enterovirus diagnostics and characterisation within and beyond Europe. *J Clin Virol*. 2018;101:11-7. https://doi.org/10.1016/j.jcv.2018.01.008

44. Khan F. Enterovirus D68: acute respiratory illness and the 2014 outbreak. *Emerg Med Clin North Am*. 2015;33(2):e19-e32. https://doi.org/10.1016/j.emc.2014.12.011

45. Messacar K, Abzug MJ, Dominguez SR. The emergence of enterovirus-D68. *Microbiol Spectr*. 2016;4(3). https://doi.org/10.1128/microbiolspec.EI10-0018-2016

46. Holm-Hansen CC, Midgley SE, Fischer TK. Global emergence of enterovirus D68: a systematic review. *Lancet Infect Dis*. 2016;16(5):e64-e75. https://doi.org/10.1016/S1473-3099(15)00543-5

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Hybrid ameloblastoma and odontogenic keratocyst in a patient with Gorlin-Goltz syndrome: case report

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ABSTRACT

Introduction: Hybrid odontogenic lesions combine characteristics of two or more lesions in a single site, and they are considered a rare condition. The occurrence of these lesions in patients with Gorlin-Goltz syndrome is even less common. Since the lesion has variable clinical aspects and multiple imaging characteristics, histopathological examination is mandatory for a definitive diagnosis. **Methods**: This article reports the case of a patient with Gorlin-Goltz syndrome, who had multiple odontogenic keratocysts in the mandible and maxilla, as well as a combined/hybrid ameloblastoma and odontogenic keratocyst lesion. **Results**: Distinct areas with histological patterns characteristic of the cyst and tumor were observed within the same lesion. **Conclusion**: This unusual but still possible diagnosis should be considered by pathologists and oral and maxillofacial surgeons when evaluating, diagnosing and treating patients with syndromes characterized by odontogenic lesions.

Keywords: ***ameloblastoma; odontogenic keratocyst; Gorlin-Goltz Syndrome; hybrid lesion***

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INTRODUCTION

Hybrid odontogenic lesions are defined by the combined histopathological characteristics of cysts and/or odontogenic tumors at the same primary site. The occurrence of hybrid lesions composed of ameloblastoma and odontogenic keratocyst are an extremely rare[1,2,3,4].

The odontogenic keratocyst, a locally invasive cystic lesion with a high recurrence rate, is one of the major criteria for the diagnosis of Gorlin–Goltz syndrome (Nevoid basal cell carcinoma syndrome)[5].

Ameloblastoma is a slow-growing, benign, odontogenic tumor that affect the posterior region of the mandible in most cases. It is an aggressive neoplasm that tends to infiltrate the bone trabeculae[6].

Usually, both odontogenic keratocyst and ameloblastoma are found on imaging and are difficult to distinguish clinically and radiographically, thus requiring histopathological examination for the final diagnosis. Hybrid odontogenic lesions, which combine histological characteristics of different lesions, present an even greater diagnostic challenge[1,2,7,8,9].

The main objective of this article was to describe an unusual case of a combined/hybrid ameloblastoma and odontogenic keratocyst in a patient with Gorlin–Goltz syndrome with multiple keratocysts in the maxilla and mandible.

CASE REPORT

Patient WCV, male, 30 years old, with a genetic diagnosis of Gorlin–Goltz syndrome [karyotype: 46, XY, del (9)(q22.1-22.33], is currently under clinical care at the Hospital de Clínicas de Porto Alegre (HCPA), presenting with several teeth with coronoradicular destruction due to dental caries. No expansive intra-buccal lesions were observed.

Panoramic radiographic examination (Figure 1) revealed four osteolytic lesions: a radiolucent lesion with undefined limits in the right maxilla,

a well-defined radiolucent lesion in the right posterior mandible, a unilocular, well-defined radiolucent lesion with a radiopaque halo in the left posterior mandible, and a radiolucent lesion with undefined limits in the anterior region of the right mandible. Considering the genetic diagnosis of Gorlin–Goltz syndrome, the diagnostic hypothesis for lesions in the mandible was multiple odontogenic keratocysts; for maxillary lesions, the hypothesis of an inflammatory apical lesion was considered to be due to the presence of teeth with extensive caries-related lesions.

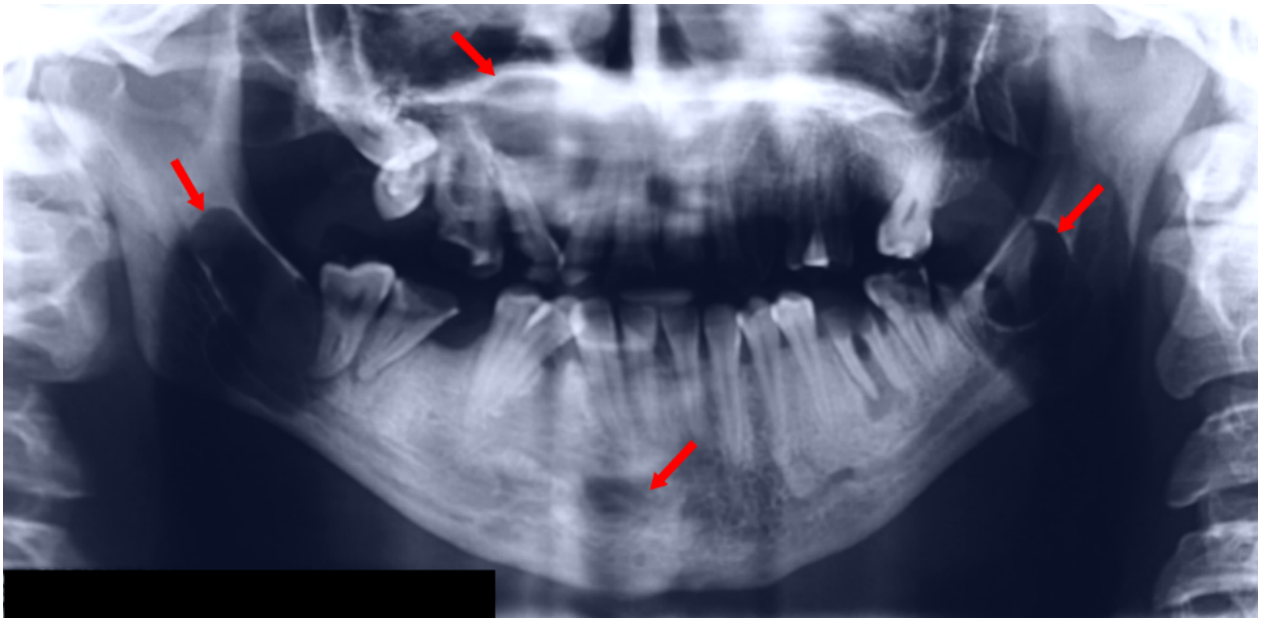

Figure 1: Panoramic radiography, showing radiolucent lesions (arrows) in the maxilla and mandible. Coronorradicular lesions caused by dental caries are present.

The patient underwent surgical intervention under general anesthesia for the total removal of the intraosseous lesions in the maxilla and mandible, as well as dental extractions. Peripheral ostectomy and liquid nitrogen cryotherapy were performed at all surgical sites as adjuvant therapies. The excised lesions were subjected to histopathological analysis.

The lesion of the right maxilla was characterized by the presence of a cystic cavity coated with a parakeratinized stratified epithelium with a few layers, a corrugated surface, and a basal layer with hyperchromatic nuclei arranged in a palisade. Fragile union between the epithelium and connective tissue, and a flat interface without epithelial crests were observed. Microscopy revealed typical characteristics of odontogenic keratocyst (Figure 2A). In a region distant from the cystic cavity, islands of odontogenic epithelium are separated by dense connective tissue. On some islands, peripheral palisading of cells can be observed; however, in most of them, the cells appear more cuboidal. No stellate reticulum pattern was observed at the center of the islands. The microscopic characteristics resembled those of basal cell ameloblastoma (Figure 2B).

The lesion located in the right posterior mandible was characterized by a cystic cavity covered by a parakeratinized stratified epithelium consisting of a few layers, with a corrugated surface and a basal layer with hyperchromatic nuclei arranged in a palisade. Fragile union between the epithelium and connective tissue, and a flat interface without epithelial crests were observed. Microscopy revealed odontogenic keratocyst characteristics (Figure 2C).

Microscopic characteristics typical of odontogenic keratocyst were observed in the lesions located in the left posterior mandible (Figure 2D). Some islands of odontogenic epithelium with basal cells and polarized nuclei, such as pre-ameloblasts, which are characteristic of ameloblastoma, were observed in another region of the same lesion. The stellate reticulum pattern at the center of the islands is not well defined (Figure 2E).

The lesion, located in the right anterior mandible, showed a cavity covered with a parakeratinized stratified epithelium and a basal layer with hyperchromatic nuclei. Epithelial cells proliferated towards the connective tissue, forming islands of odontogenic epithelium in a solid pattern (Figure 2F). This feature has been reported in cases of isolated odontogenic keratocyst and in patients with Gorlin–Goltz syndrome.

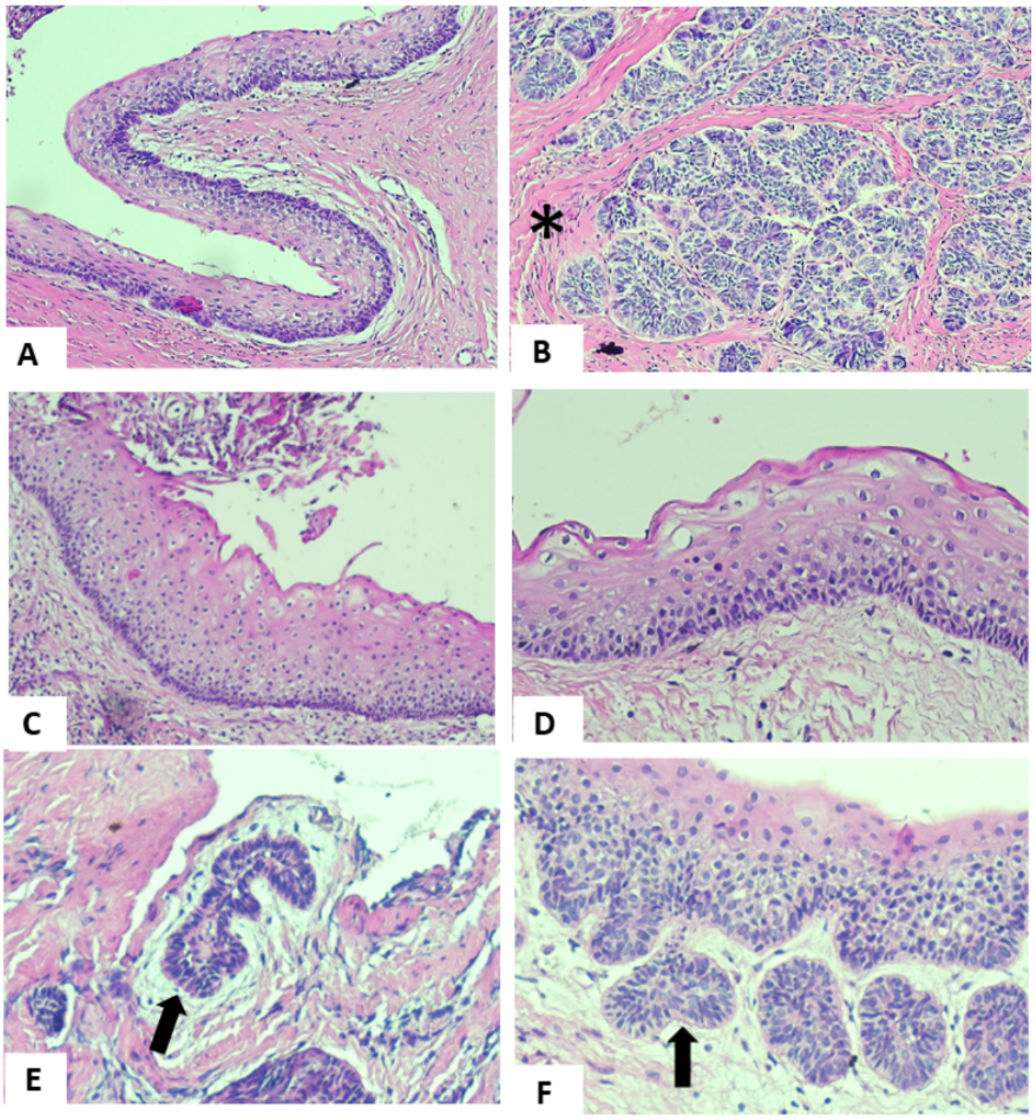

Figure 2: Microscopic aspect of the lesions. A and B: Lesion in the right maxilla; A: microscopic characteristics of keratocysts; B: islands of odontogenic epithelium between fibrous connective tissue (*), characteristic of ameloblastoma. C: Microscopy of the lesion in the right posterior mandible shows an odontogenic keratocyst. D and E: Lesion in the left posterior mandible. D: An odontogenic keratocyst; E: islands of the odontogenic epithelium in the connective tissue capsule, presenting high peripheral cells (arrows) with ameloblastomatous characteristics; F: Lesion in the anterior right mandible, showing the proliferation of islands of odontogenic epithelial cells (arrows) from the lining epithelium of the odontogenic keratocyst.

DISCUSSION

Hybrid odontogenic lesions are extremely rare[1]. Hybrid lesions composed of ameloblastoma and odontogenic keratocyst have been described in the literature as keratocyst with ameloblastic alterations consisting of reverse polarity and apical vacuolization[1,2]. There is an isolated case of simultaneous occurrence of both lesions in the published English literature, but as completely distinct events: odontogenic keratocyst on the left side of the mandible and ameloblastoma on the right side of the mandible[5]. Neuman et al.[1] described two cases of hybrid odontogenic lesions, one of which was an odontogenic keratocyst presenting as odontogenic epithelial islands with ameloblastic characteristics far from the cystic cavity, as in the present case.

Because the patient was diagnosed with Gorlin–Goltz syndrome, imaging examinations showing osteolytic lesions in the maxilla and mandible were preoperatively interpreted as odontogenic keratocyst[7]. The lesions located in the right posterior and right anterior mandible were histopathologically diagnosed as odontogenic keratocyst with a characteristic histopathological pattern. Histopathological examination

of the lesions excised from the right maxilla and left posterior mandible revealed cystic lesions with characteristics of odontogenic keratocyst and islands of odontogenic epithelium in the stroma. In this case report, the right maxilla presented with two distinct lesions at the same site, which were diagnosed using microscopy as hybrid lesion. The lesion in the left posterior mandible presented as a few islands with an ameloblastic pattern, which may have been an ameloblastomatous alteration in the islands of the odontogenic epithelium from the odontogenic keratocyst. These characteristics have also been described individually or together in other reports on hybrid lesions[1,7,9,10].

Odontogenic keratocyst and ameloblastoma may present with similar clinical and imaging features, requiring histopathological examination for a final diagnosis[3,8,9]. The literature discusses whether hybrid odontogenic lesions originate concomitantly from a common pluripotent epithelial cell, or represent the coexistence of two distinct lesions[1].

The patient was treated with enucleation of the lesions, followed by adjuvant therapy (peripheral ostectomy and cryotherapy). This therapeutic option is recommended for hybrid lesions, because recurrence is rare[7]. The patient was kept under clinical and imaging monitoring, with no signs of lesion recurrence at the five-year postoperative follow-up.

In conclusion, the occurrence of a combined/hybrid odontogenic lesion composed of ameloblastoma and odontogenic keratocyst in a patient with Gorlin–Goltz syndrome with multiple odontogenic keratocystic lesions is rare, and little is about the etiology of the lesion, whether they were originally two separate lesions coexisting or an ameloblastic transformation of an odontogenic keratocyst, as suggested by some microscopic fields (Figure 2F). The diagnosis of hybrid odontogenic lesions should be considered and presents a challenge for oral and maxillofacial surgeons and oral pathologists; because their histogenesis is controversial, imaging results may vary, and clinical behavior is still poorly understood.

REFERENCES

1. Neuman AN, Montague L, Cohen D, Islam N, Bhattacharyya I. Report of two cases of combined odontogenic tumors: ameloblastoma with odontogenic keratocyst and ameloblastic fibroma with calcifying odontogenic cyst. Head Neck Pathol. 2015;9(3):417-20.
2. Gupta RK, Dugal AG, Pawar SR, Khandelwal SG, Iyengar A. A rare simultaneous occurrence of odontogenic keratocyst and unicystic ameloblastoma in mandible: a case report. J Clin Diagn Res. 2016;10(8):ZD01-ZD4.
3. Rosa ACG, Soares AB, Furuse C, Lima SRR, de Araújo VC, Passador-Santos F. A combined epithelial odontogenic tumor? A 7-year follow-up case. Head Neck Pathol. 2017;11(4):519-24.
4. Brannon RB. The odontogenic keratocyst. A clinicopathologic study of 312 cases. Part I. Clinical features. Oral Surg Oral Med Oral Pathol. 1976;42(1):54-72.
5. Silva LP, Rolim LS, Silva LA, Pinto LP, Souza LB. The recurrence of odontogenic keratocysts in pediatric patients is associated with clinical findings of Gorlin-Goltz Syndrome. Med Oral Patol Oral Cir Bucal. 2020;25(1):e56-e60.
6. Effiom OA, Ogundana OM, Akinshipo AO, Akintoye SO. Ameloblastoma: current etiopathological concepts and management. Oral Dis. 2018;24(3):307-16.
7. Pontes FSC, Mosqueda-Taylor A, Souza LL, Paula LP, Batista LAL, Rodrigues-Fernandes CI, Paiva e Costa AM, Abreu MC, Gomez RS, de Oliveira EM, Fonseca FP, Rahimi S, Brennan PA, Pontes HAR. Hybrid odontogenic lesions: A systematic review of 203 cases reported in the literature. J Oral Pathol Med. 2022;51(1):5-12.
8. Kreppel M, Zöller J. Ameloblastoma-Clinical, radiological, and therapeutic findings. Oral Dis. 2018;24(1-2):63-6.
9. Alves DBM, Tuji FM, Alves FA, Rocha AC, Santos-Silva ARD, Vargas PA, Lopes MA. Evaluation of mandibular odontogenic keratocyst and ameloblastoma by panoramic radiograph and computed tomography. Dentomaxillofac Radiol. 2018;47(7):1-7.
10. Siar CH, Ng KH. 'Combined ameloblastoma and odontogenic keratocyst' or 'keratinising ameloblastoma'. Br J Oral Maxillofac Surg. 1993;31(3):183-6.

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Gastroenterite Eosinofílica: Um relato de caso

Eosinophilic Gastroenteritis: A case report

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RESUMO

Introdução: As Gastroenterites Eosinofílicas (GE) são doenças crônicas imunomediadas caracterizadas histologicamente por um aumento de eosinófilos nas diferentes camadas teciduais do trato gastrointestinal. As GE se referem a um grupo de condições, incluindo a esofagite eosinofílica (EE), a gastrite eosinofílica (GE), a enterite eosinofílica (ENE) e a colite eosinofílica (CE). A prevalência da GE nos Estados Unidos é estimada em 22 a 28 por 100.000 pessoas e é maior em crianças <5 anos. Quando presentes em adultos, são mais comuns na terceira e quinta décadas de vida, tendo proporção igual entre homens e mulheres. Sugere-se um componente alérgico na fisiopatogenia das doenças, com 50 a 70% dos pacientes apresentando condições atópicas concomitantes.

Método: relatamos um caso de uma paciente com gastroenterite eosinofílica, baseado na história da paciente e registro de prontuário.

Resultado: O caso descrito demonstra a importância do conhecimento dos critérios clínicos para o diagnóstico dessa patologia.

Conclusão: O tratamento equivocado prolonga o quadro clínico e potencializa o aparecimento de possíveis complicações com impacto negativo na qualidade de vida.

Palavras-chave: *Eosinofilia, gastroenterite, dor abdominal*

ABSTRACT

Introduction: Eosinophilic Gastroenteritis (EG) are chronic immune-mediated diseases histologically characterized by an increase in eosinophils in the different tissue layers of the gastrointestinal tract. EG refers to a group of conditions including eosinophilic esophagitis (EE), eosinophilic gastritis (EG), eosinophilic enteritis (ENE) and eosinophilic colitis (EC). The prevalence of EG in the United States is estimated at 22 to 28 per 100,000 people and is higher in children <5 years old. When present in adults, they are more common in the third and fifth decades of life, with equal proportion between men and women. An allergic component is suggested in the pathophysiology of the diseases, with 50 to 70% of patients having concomitant atopic conditions.

Methods: we report a case of a patient with eosinophilic gastroenteritis, based on the patient's history and medical records.

Results: This case demonstrates the importance of knowing the clinical criteria for the proper diagnosis of this pathology.

Conclusion: The wrong treatment prolongs the clinical condition and enhances the appearance of possible complications with a negative impact on quality of life.

Keywords: *Eosinophilia, gastroenteritis, abdominal pain*

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APRESENTAÇÃO DO CASO

Homem, 25 anos, pardo, bancário, sem comorbidades prévias, sem histórico de atopias. Procurou atendimento no departamento de emergência com relato de dor epigástrica de forte intensidade, sem irradiação e desencadeada cerca de 1h após refeição copiosa (carne de gado, massa e batata inglesa). Não obteve alívio da dor com uso de analgésicos simples no domicílio.

Referiu que fez uma viagem recente à praia. Quanto ao histórico pregresso, relatou que fez tratamento para *Helicobacter pylori* em 2017 e que possuía histórico familiar de colelitíase.

Ao exame físico, o paciente era normolíneo, IMC de 23,3kg/m², os sinais vitais eram estáveis e ele possuía dor à palpação superficial da região epigástrica, porém sem evidência de contratura de defesa e o sinal de Murphy negativo. Foi solicitado exames iniciais, que evidenciaram eosinofilia - Tabela 1.

Foi optado por internação hospitalar, considerando necessidade de maior investigação diagnóstica e devido à refratariedade ao uso de analgésicos no departamento de emergência (dipirona e buscopam).

O paciente persistiu com episódios de dor abdominal, principalmente no período pós-prandial (recebendo dieta branda: gelatina, sopa, caldo, pão torrado, carne moída e vegetais cozidos). Descrevia a dor com característica de "facada". No segundo dia da internação, evoluiu com diarreia amarelada, de grande volume, fezes Bristol 6, três episódios/dia. Sendo assim, foi solicitada a coleta de 3 amostras de fezes para análise parasitológica e coprocultura. Após, optado por início empírico de Ciprofloxacino e Metronidazol.

Foi realizada esofagogastroduodenoscopia com biópsia, que demonstrou erosões em bulbo e 2ª porção duodenal (Figura 1).

Tabela 1: Exames laboratoriais

	RESULTADO	VALOR DE REFERÊNCIA
HEMOGLOBINA	15,9 g/dL	12-18 g/dL
HEMATÓCRITO	46%	39,2-49%
VOLUME CORPUSCULAR MÉDIO (VCM)	87 fL	81,7-95,3 fL
HEMOGLOBINA CORPUSCULAR MÉDIA (HCM)	30 pg	27,7-32,7 pg
RDW	12,50%	11,8-14,1%
LEUCÓCITOS	1.330/µL	4.000-11.000/µL
SEGMENTADOS	7.172/uL	2.000 a 7.000/ µL
EOSINÓFILOS	2.279/uL	50-400/uL
LINFÓCITOS	2.706/µL	1.000-4.500/µL
PLAQUETAS	240.000/mm³	140.000-450.000/mm³
UREIA	24mg/dL	10-45mg/dL
CREATININA	1,06mg/dL	0,7-1,3mg/dL
PROTEÍNA C REATIVA	3mg/L	<3mg/L
EPF EM 3 AMOSTRAS	Negativo	Negativo
COPROCULTURA	Sem crescimento de microrganismos	Sem crescimento de microrganismos

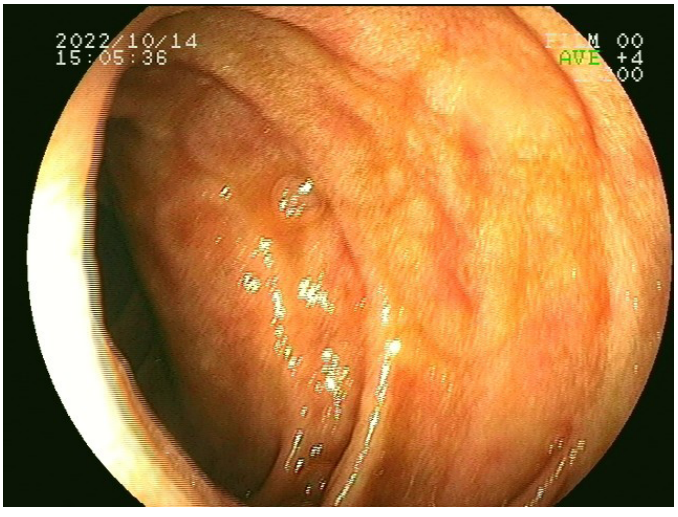

Figura 1: Esofagogastroduodenoscopia evidenciando erosões e enantema em bulbo e segunda porção duodenal

O anatomopatológico evidenciou infiltrado eosinofílico em bulbo duodenal (20 eosinófilos por campo de grande aumento na lâmina própria e 7 eosinófilos intraepitelias) e segunda porção duodenal (30 eosinófilos por campo de grande aumento na lâmina própria e 14 eosinófilos intraepiteliais) (Figura 2). Os fragmentos da mucosa gástrica possuíam alterações inflamatórias mínimas e ausência de eosinófilos.

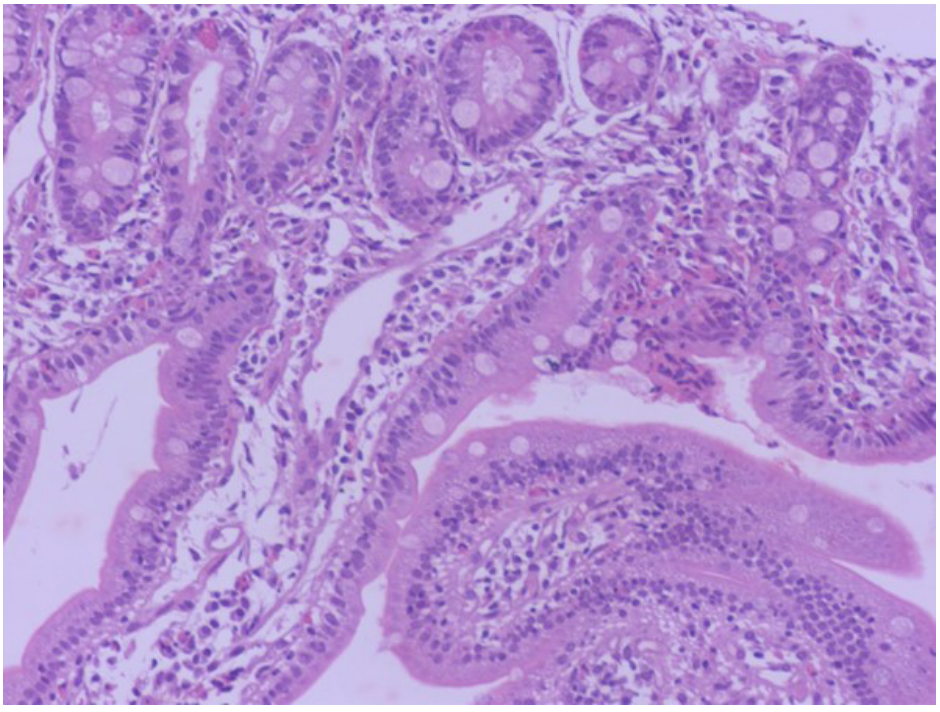

Figura 2: Anatomopatológico evidenciando infiltrado eosinofílico em bulbo duodenal e segunda porção duodenal

Englobando o quadro clínico do paciente, com os achados dos exames laboratoriais e de patologia das biópsias duodenais, foi estabelecido o diagnóstico de enterite eosinofílica. Sendo assim, foi iniciado tratamento com corticoesteroide (prednisona 20mg/dia) e o paciente evoluiu com remissão do quadro gastrintestinal e resolução da eosinofilia.

DISCUSSÃO

Todo o trato gastrointestinal, do esôfago ao cólon, pode ser afetado em pacientes com doenças gastrointestinais eosinofílicas. A invasão eosinofílica determina sintomas de acordo com o órgão e a camada afetada[1].

A doença restrita à mucosa traduz sintomas inespecíficos, como dor abdominal, plenitude, náuseas e vômitos, cerca de um terço dos pacientes com essa condição apresentam perda de peso associada. Extensos acometimentos de intestino delgado podem cursar com desnutrição e déficit de crescimento[2].

Quando há acometimento da camada muscular, observa-se um espessamento da parede e predominam sintomas de dismotilidade, distensão abdominal e até mesmo obstrução. Casos mais avançados podem cursar com perfuração gástrica, do intestino delgado e, mais raramente, do cólon[2,3].

Pacientes com doença eosinofílica que acomete a serosa podem apresentar ascite e derrame pleural, associados ou não a sintomas gastrintestinais. A característica principal na análise de líquido ascítico é a presença de numerosa de eosinófilos[2].

As desordens eosinofílicas podem ser divididas em primárias ou secundárias. Fatores de risco ambientais e genéticos podem desencadear a patologia, sendo que existe forte associação com exposição a alérgenos alimentares e antígenos ambientais. Há diversas causas associadas aos distúrbios secundários, como: alergias alimentares, infecções bacterianas ou parasitárias, doença celíaca, doença inflamatória intestinal, doenças do tecido conjuntivo, vasculites sistêmicas, neoplasias mieloproliferativas, transplantes de órgãos sólidos e hipersensibilidade a medicamentos[3].

A resposta de hipersensibilidade desempenha um papel importante na patogênese da doença, visto que muitos pacientes com gastroenterite eosinofílica apresentam história de atopias sazonais, alergias alimentares, eczema, asma e níveis séricos elevados de IgE[3,4].

Embora exista essa forte correlação entre gastroenteropatia eosinofílica com atopias, com estatísticas sugerindo concomitância de componente alérgico em 50 a 70% dos casos, com IgE elevado especialmente em crianças, a fisiopatologia da GE e ENE ainda não é completamente compreendida[5,6]. No entanto, quando sugere-se uma dieta livre de alérgenos, observa-se uma importante melhora sintomática, endoscópica e histológica. No presente relato, há eosinofilia em sangue periférico e história negativa para atopias[7].

O acometimento exclusivo do intestino delgado pode cursar com enteropatia perdedora de proteínas e hipoalbuminemia, aumento da excreção fecal de gorduras, má absorção de carboidratos com teste de D-xilose anormal e anemia, devido a absorção deficitária de ferro ou sangramento digestivo[8].

A eosinofilia em sangue periférico, na presença de clínica compatível, corrobora com o diagnóstico, predominantemente em doença que acomete a mucosa ou a serosa, sendo normal em 20% dos casos[4].

Os exames de imagem são inespecíficos e pouco sensíveis, podendo revelar apenas espessamento de parede ou "dente de serra" em intestino delgado. Tomografia e ressonância magnética de abdome não foram realizados neste paciente[8].

Dessa forma, o diagnóstico de gastroenteropatia eosinofílica é feito com biópsia revelando infiltrado eosinofílico ou líquido ascítico eosinofílico na ausência de outras causas de eosinofilia gastrointestinal. No paciente em questão, após coprocultura e EPF 3 amostras negativos, optou-se por realização de esofagogastroduonenoscopia com biópsias, cujo anatomopatológico evidenciou infiltrado eosinofílico em bulbo e segunda porção duodenal.

REFERÊNCIAS

1. Matsushita M, Hajiro K, Morita Y, Takakuwa H, Suzaki T. Eosinophilic gastroenteritis involving the entire digestive tract. *Am J Gastroenterol*. 1995;90(10):1868-70.
2. Talley NJ, Shorter RG, Phillips SF, Zinsmeister AR. Eosinophilic gastroenteritis: a clinicopathological study of patients with disease of the mucosa, muscle layer, and subserosal tissues. *Gut*. 1990;31(1):54-8.
3. Oh H., Chetty R. Eosinophilic gastroenteritis: a review. J. Gastroeterol. 2007 May 43:741-750.
4. Chehade M, Magid MS, Mofidi S, Nowak-Wegrzyn A, Sampson HA, Sicherer SH. Allergic eosinophilic gastroenteritis with protein-losing enteropathy: intestinal pathology, clinical course, and long-term follow-up. *J Pediatr Gastroenterol Nutr*. 2006;42(5):516-21.
5. Ko HM, Morotti RA, Yershov O, Chehade M. Eosinophilic gastritis in children: clinicopathological correlation, disease course, and response to therapy. *Am J Gastroenterol*. 2014;109(8):1277-85.
6. Higuchi T, Tokunaga M, Murai T, Takeuchi K, Nakayama Y. Elemental diet therapy for eosinophilic gastroenteritis and dietary habits. *Pediatr Int*. 2022;64(1):e14894.
7. Cello JP. Eosinophilic gastroenteritis – a complex disease entity. Am J Med. 1979;67(6):1097-104.
8. MacCarty RL, Talley NJ. Barium studies in diffuse eosinophilic gastroenteritis. Gastrointest Radiol. 1990;15(1):183-7.

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O desfecho da ventilação não invasiva em pacientes acometidos pela COVID-19

The outcome of non-invasive ventilation in patients affected by COVID-19

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RESUMO

Introdução: A doença do coronavírus (COVID-19) espalhou-se rapidamente pelo mundo e foi considerada uma pandemia no dia 11 de março de 2020. O desfecho de saúde de indivíduos acometidos varia, e, de 5 a 20% se tornam graves. O uso da ventilação não invasiva (VNI) mesmo sem consenso inicial teve crescimento como terapia de primeira linha. O objetivo deste estudo é investigar o desfecho dos pacientes com COVID-19 que utilizaram a VNI como terapia de primeira linha, relacionar pressão arterial de oxigênio (PaO_2) inicial com o resultado encontrado, descrever o perfil epidemiológico dos pacientes e avaliar o desfecho da internação na unidade de terapia intensiva (UTI).

Métodos: Estudo observacional, transversal, retrospectivo, realizado através da análise de prontuários de pacientes acometidos pela COVID-19 atendidos na UTI de um hospital do Vale dos Sinos, entre janeiro e julho de 2021.

Resultados: Foram incluídos 49 pacientes, 63,3% do sexo masculino, com idade de 47,5 ± 12,9 anos, 96% apresentou comorbidades e somente 36,7 % melhorou apenas com o uso da VNI, o que tem forte relação (p = 0,007) com o nível inicial de PaO2. Obteve-se uma taxa de alta da UTI de 67,3%.

Conclusão: Pacientes infectados com COVID-19 podem se beneficiar do uso da VNI, desde que a escolha seja feita de forma segura levando em consideração a gravidade da hipoxemia.

Palavras-chave: *Ventilação Mecânica; Ventilação não invasiva; COVID-19; Cuidado intensivo*

ABSTRACT

Introduction: The coronavirus disease (COVID-19) has spread rapidly around the world and has been declared a pandemic on March 11, 2020. The health outcome of affected individuals varies, and from 5 to 20% becomes severe. The use of non-invasive ventilation (NIV), even without an initial consensus, grew as a first-line therapy. The objective of this study is to investigate the outcome of patients with COVID-19 who used NIV as a first-line therapy, relate initial arterial oxygen pressure (PaO_2) with the result found, describe the epidemiological profile of patients and evaluate the outcome of hospitalization in the intensive care unit (ICU).

Methods: Observational, cross-sectional, retrospective study, carried out through the analysis of medical records of patients affected by COVID-19 treated in the ICU of a hospital in Vale dos Sinos, between January and July 2021.

Results: 49 patients were included, 63.3% male, aged 47.5 ± 12.9 years, 96% had comorbidities and only 36.7% improved only with the use of NIV, which is strongly related (p = 0.007) with the initial PaO2 level. An ICU discharge rate of 67.3% was obtained.

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Conclusion: Patients infected with COVID-19 may benefit from the use of NIV, as long as the choice is made safely, taking into account the severity of hypoxemia.

Keywords: *Mechanical Ventilation; Non-Invasive Ventilation; COVID19; Intensive Care.*

INTRODUÇÃO

O coronavírus (COVID-19) é uma infecção viral do trato respiratório, altamente contagiosa, que foi documentada pela primeira vez em dezembro de 2019 na China[1], espalhou-se rapidamente pelo mundo, chegando ao Brasil em de fevereiro de 2020[2]. Foi considerada uma pandemia e emergência internacional pela Organização Mundial da Saúde no dia 11 de março deste mesmo ano[3]. O desfecho de saúde dos indivíduos infectados varia de casos assintomáticos ou leves, até doença grave e óbito[4,5]. É documentado que entre 5 e 20% dos pacientes evoluem para forma grave da doença desenvolvendo a Síndrome do Desconforto Respiratório Agudo (SDRA) precisando de hospitalização, oxigenoterapia, e em alguns casos uso de ventilação mecânica (VM)[1,6,7].

Ainda não existe tratamento medicamentoso para pacientes acometidos pela doença, sendo então a oxigenoterapia a principal intervenção para reverter hipoxemia, incluindo ventilação mecânica, que pode ocorrer de maneira invasiva ou não[7,8]. A ventilação mecânica não invasiva (VNI) mesmo sem um consenso sobre sua aplicação, foi utilizada em alguns casos como terapia de primeira linha, almejando alívio dos sintomas de dispneia, visando aumento de pressão arterial de oxigênio (PaO_2), conforto e redução da necessidade de intubação orotraqueal (IOT) e suas complicações[5,9].

Apesar fortes recomendações desta terapêutica para pacientes com agudização de doenças obstrutivas crônicas ou edema pulmonar cardiogênico[10,11], na insuficiência respiratória hipoxêmica aguda de origem não cardiogência os benefícios podem ser questionados ao analisar a gravidade da hipoxemia do paciente[5,12-14]. Sendo assim, o objetivo deste estudo é investigar o desfecho dos pacientes com COVID-19 que utilizaram a VNI como terapia de primeira linha, relacionar PaO2 inicial com o resultado encontrado, descrever o perfil epidemiológico dos pacientes e avaliar o desfecho da internação na UTI.

MÉTODOS

Desenho e população do estudo

Trata-se de um estudo observacional, transversal, retrospectivo, realizado com pacientes acometidos pela COVID-19 que utilizaram a VNI como terapia de primeira linha, atendidos na Unidade de Terapia Intensiva (UTI) de um hospital do Vale dos Sinos, entre janeiro e julho de 2021.

Foram incluídos no estudo todos os pacientes internados na UTI COVID-19, com idade superior a 18 anos e diagnóstico de COVID-19 que realizaram a VNI com qualquer interface como terapia de primeira linha. Pacientes que não apresentaram teste positivo para COVID-19, foram submetidos inicialmente a IOT, ou a VNI após 15 dias de sintomas foram excluídos da amostra.

Medidas e procedimentos do estudo

A coleta foi realizada através do acesso aos prontuários eletrônicos disponibilizados pelo hospital. Foram registrados dados referentes às seguintes variáveis: idade; sexo; comorbidades; número de comorbidades; data de internação; vacinação; data de início dos sintomas, data de diagnóstico da COVID-19; data de início da VNI; PaO2 inicial; dias utilizando a VNI; desfecho da VNI; desfecho final da internação na UTI.

No presente estudo, a definição do desfecho da VNI é definida como melhorada apenas com o uso da VNI quando não houve a necessidade da instuir ventilação mecanica invasiva (VMI), e falha, quando o paciente evolui para o uso da VMI. Já o desfecho final corresponde a alta da UTI ou óbito.

Os critérios para o diagnóstico de COVID-19 foram resultado positivo do teste de RT-PCR em swabs nasais e faríngeos, achados de TC de tórax consistentes com COVID-19 e/ou diagnóstico clínico firme de COVID-19 feito em ambiente hospitalar.

Ética

O presente estudo segue os preceitos éticos estabelecidos pela resolução n° 466/2012 do Conselho Nacional de Saúde, foi submetido e aprovado pelo Núcleo Municipal de Educação em Saúde Coletiva (NUMESC), do Município de Novo Hamburgo e pelo Comitê de Ética e Pesquisa da Universidade Feevale conforme o parecer número CAAE - 87870318.9.0000.5348

Metodologia Estatística

A estatística descritiva foi utilizada para apresentar os resultados através das frequências absolutas (n) e relativas (%), valores mínimos

e máximos, médias aritméticas e respectivos desvios- padrão. A modelagem da classificação para o desfecho da VNI foi obtida através de Regressão Logística Binomial, método “stepwise forward”. Para examinar associações entre o desfecho da VNI e a prevalência de comorbidades foi utilizado o Qui-Quadrado ($\chi 2$). O Teste T de Student foi utilizado para comparar os níveis da PaO2 no início da VNI entre o resultado do desfecho na VNI. Os procedimentos estatísticos foram executados no software IBM® SPSS® (Versão 26.0), adotando nível de significância em $p \leq 0,050$.

RESULTADOS

Foram analisados todos os prontuários registrados da UTI no período entre janeiro e julho de 2021, totalizando 216 pacientes, destes, 2 pacientes não apresentaram teste positivo para COVID-19, 12 não necessitaram de suporte ventilatório além da oxigenoterapia, e 144 foram submetidos a VMI como suporte ventilatório inicial. Totalizando 58 pacientes submetidos inicialmente a VNI, porém 9 foram excluídos por iniciarem com a técnica após 15 dias de início dos sintomas (Figura 1).

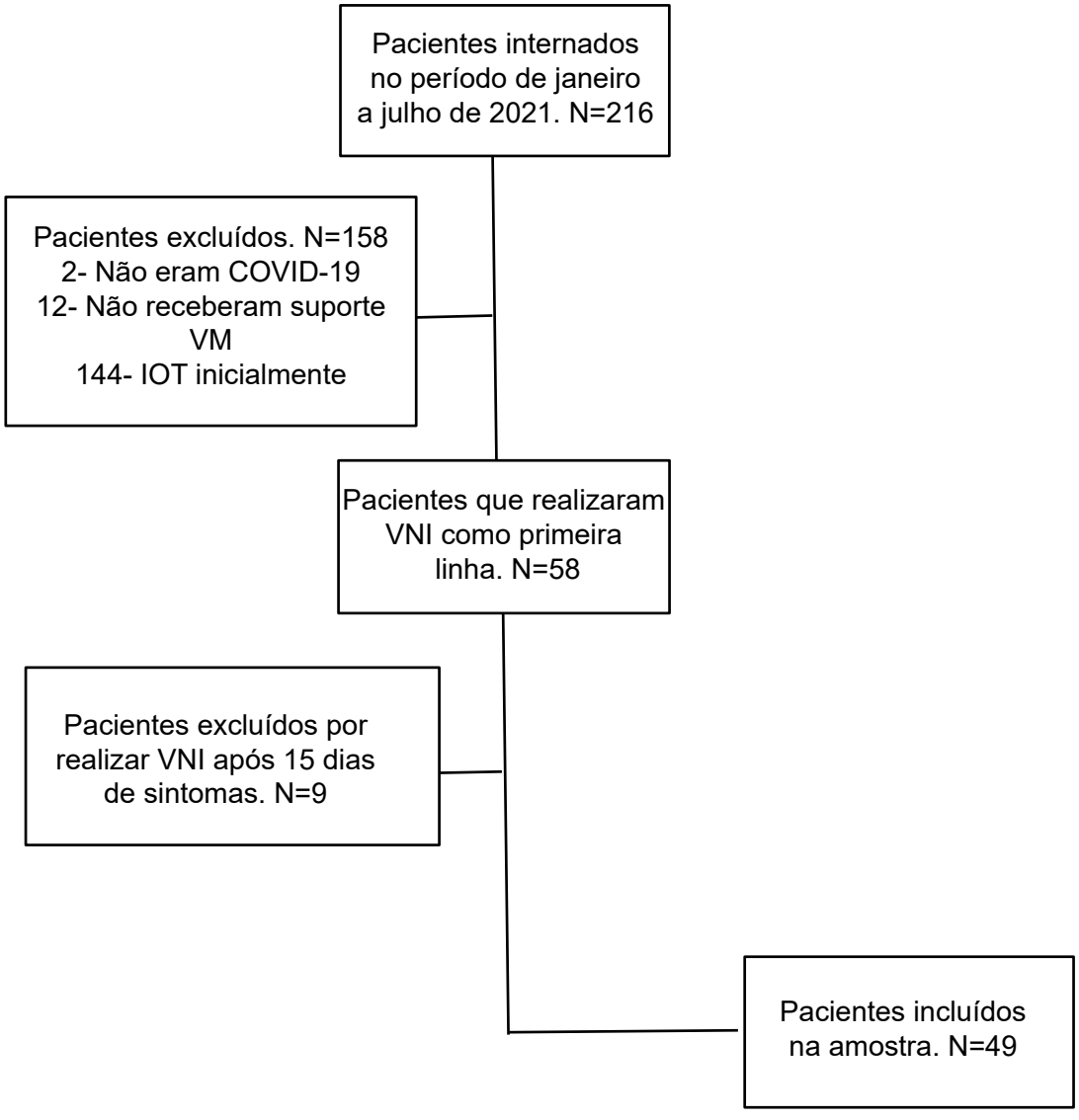

Figura 1: Fluxograma de inclusão dos pacientes no estudo. VM: ventilação mecânica; VNI: ventilação mecânica não invasiva; IOT: intubação orotraqueal.

Dos 49 pacientes que foram incluídos no estudo, 31 (63,3%) eram do sexo masculino. A média de idade foi de 47,5 ± 12,9 anos. A maioria (87,8%) não realizou nenhuma dose da vacina. E 96% dos pacientes apresentaram pelo menos um tipo de comorbidade, sendo a obesidade a mais frequente (59,6%). A Tabela 1 elucida o perfil dos pacientes, e a Tabela 2 demonstra todas as comorbidades encontradas.

Tabela 1: Perfil da amostra (n = 49).

Variáveis	Resultados
Sexo n (/%)	
Masculino	31 (63,3)
Feminino	18 (36,7)
Idade anos (DP)	47,5 ± 12,9
Vacina COVID-19 n (/%)	
Não	43 (87,8)
Sim	6 (12,2)
Comorbidades n (/%)	
Sim	47 (96)
Não	2 (4)

Tabela 2: Comorbidades (n = 47).

Variável	Categorias	n	%
Comorbidade [1]	Obesidade	28	59,6
	Hipertensão arterial sistêmica	20	42,6
	Sobrepeso	12	25,5
	Ex-Tabagista	9	19,1
	Diabetes Mellitus	8	17,0
	Colecistectomia	3	6,4
	Asma	3	6,4
	Tabagista	3	6,4
	Outras (apenas 1 citação cada)	16	34,0

[1] A soma excede 100% em função das múltiplas respostas. Outras comorbidades (apenas uma citação cada): etilismo, cirrose hepática, gota, dislipidemia, IAM previo, IRC agudizada, DM tipo I, neoplasia na próstata, nódulo mamário, apendicectomia com complicações, síndrome do pânico, câncer mediastino, DM gestacional, transtorno bipolar, tromboembolismo pulmonar e válvula metálica aórtica).

Em relação ao desfecho da VNI notou-se que a minoria (36,7%) dos pacientes evoluiu de forma melhorada (Figura 2). Esse resultado parece ter forte relação com o nivel inicial de PaO2 do paciente ao ser submetido a terapia, aqueles que possuiam PaO_2 mais baixas apresentaram mais chances (p= 0,007) de evoluir para IOT (Figura 3).

Quanto a associação da IOT e a presença de comorbidades, não há resultado significativo para o desfecho (p=0,068), porém em função do p-value estar muito próximo da significância, observa-se uma tendência, pacientes que não apresentam comorbidades têm maiores chances de melhorarem sem a necessidade de IOT (Tabela 3).

Quando avaliado o desfecho final da internação na UTI a maioria (67,3%) dos pacientes incluidos no estudo obteve alta, entretanto ao estratificar esses dados e analisar os pacientes que evoluiram para falha na VNI, indentifica-se um alto indice de mortalidade chegando a quase metade (48,4%) da população (Tabela 4).

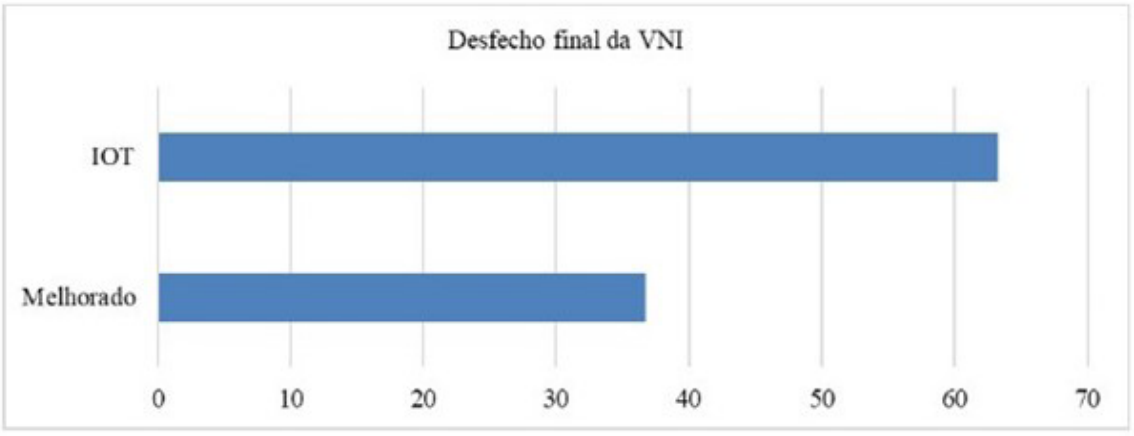

Figura 2: Desfechos da ventilação não invasiva.

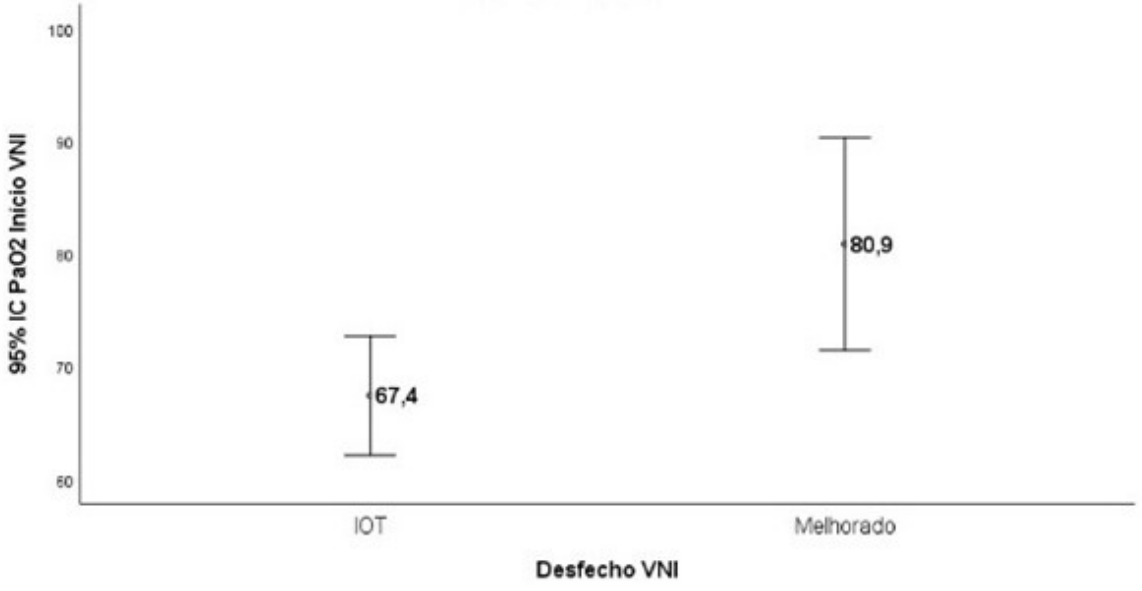

Figura 3: PaO_2 (em mmHg) no início da VNI em função do desfecho (n = 49; p = 0,007).

Tabela 3: Desfecho em IOT em função da prevalência de comorbidades.

		Comorbidades				Total	
		Presente		Ausente			
		N	%	n	%	n	%
Desfecho VNI	IOT	31_a	66,0	0_a	0,0	31	
	Melhorado	16_a	34,0	2_a	100,0	18	
Total		47		100,0	2	100,0	49

χ^2 = 3,59; gl = 1; p = 0,068. Cada letra de subscrito indica um subconjunto de sexo categorias cujas proporções da coluna não se diferem significativamente umas das outras em $p \leq 0,05$.

Tabela 4: Desfechos da internação

Desfecho	n	%
Total (49)		
Alta	33	67,3
Óbito	18	36,7
IOT (31)		
Alta	16	51,6
Óbito	15	48,4
Melhorados com VNI (18)		
Alta		100
Óbito		0

DISCUSSÃO

No presente estudo observamos que 63% da amostra é composta por homens, o que é proporcional aos resultados encontrados em pesquisas que traçam o perfil epidemiológico de pacientes com COVID-19[2,6,15,16], e fundamentado pelos resultados referentes ao perfil observado em pacientes graves submetidos ao uso de VNI[13,14]. Em relação à idade, obteve-se uma média de 47,5 anos, o que se diverge da literatura[15,17,18], onde a população estudada apresentou em média entre 50 e 60 anos. A minoria dos indivíduos (12,2%) era vacinada, o que pode ser justificado pela baixa disponibilidade de insumos no mercado naquele período e a necessidade da divisão em grupos prioritários[19].

Pacientes com comorbidades têm predisposição a se tornarem mais graves, e o desenvolvimento da SDRA em pacientes com COVID-19 tem forte relação com a presença de doenças crônicas não transmissíveis[4,5,15,18]. Alguns autores[4,5,20] relatam que indivíduos com COVID-19 hospitalizados apresentam principalmente alteração no índice de massa corporal, diabetes mellitus, e hipertensão arterial sistêmica (HAS). Nosso estudo se assemelha com os resultados discutidos, pois 96% da amostra apresentou pelo menos um tipo de comorbidade, sendo a obesidade predominante seguida da HAS, e aqueles que não apresentaram comorbidades (4%) evoluíram sem necessidade de IOT.

As indicações do uso da VNI para tratar pacientes com SDRA são limitadas, e seu uso em pacientes com COVID-19 apresentou muitas discrepâncias, devido principalmente à falta de recomendações nas diretrizes clínicas, e repercussões conflitantes da sua aplicação durante outras pandemias[5,11,17,21,22]. Apesar dos resultados controversos, seu uso como tratamento inicial teve uma crescente com o aumento dos casos de COVID-19 e sobrecarga do sistema, no desejo de aliviar a hipoxemia e a dispneia dos pacientes e reduzir a necessidade de IOT e suas complicações[5,22].

Em nosso estudo, analisamos se o uso da VNI como terapia inicial evitou a necessidade de IOT. A técnica foi aplicada nos pacientes independentemente da gravidade da hipoxemia, e pode-se afirmar que existe forte relação desta variável com o desfecho. Pacientes que apresentaram PaO_2 acima de 80mmHg tiveram um desfecho favorável enquanto os que possuíam PaO_2 de 60mmHg necessitaram de IOT.

É conhecido pela literatura que PaO_2 abaixo de 60 mmHg indica hipoxemia grave[23,24], o que fundamenta os resultados obtidos nesse estudo e corrobora com afirmações encontradas na literatura relacionando a gravidade da hipoxemia com o maior risco de falha da VNI em pacientes com SDRA5,9,22,25.

Constatou-se nesse estudo que o uso da VNI como terapia de primeira linha apresentou altos índices de falha, chegando a 63,7% dos pacientes, no local estudado existiu limitação de materiais e excasses de profissionais especializados, a mascara oronasal foi a única disponivel, e a equipe assistencial, em sua maioria, foi contratada em carater emergencial e recebeu breve treinamento sobre o uso da terapia durante o curso da pandemia, o que pode ter relação com esse desfecho. Esses resultados se assemelham com o encontrado em outro estudo que avaliou pacientes com SDRA moderada ou grave submetidos a VNI, e constatou que 56% indivíduos falharam[9], assim como os resultados encontrados em pacientes acometidos com pneumonia grave pelo vírus influenza A, onde somente 40,7% dos pacientes obtiveram sucesso com a terapia. Isso pode ser justificado pela severidade da hipoxemia, corroborando com os resultados achados por Bellani et al.[25], que realizaram um grande estudo sobre o uso da VNI em pacientes com SDRA e concluíram que pacientes com hipoxemia grave apresentam alta probabilidade de falha e aumento de mortalidade.

As estratégias não invasivas são utilizadas visando evitar a IOT e seus eventos adversos, contudo, existe grande preocupação sobre a falha do tratamento e a intubação tardia contribuírem para resultados

ruins[22,26,6] é afirmado por estudos que esse desfecho está associado a um aumento substancial no risco de morte, com mortalidade maior do que para SDRA grave tratada com VMI[9,25], uma das explicações para esse desfecho é que pacientes graves na tentativa de manter a homeostase podem iniciar com ciclos respiratórios exacerbados e vigorosos, aumentando forças trans pulmonares o que pode resultar em aumento da inflamação e suceder em biotrauma[17]. Bai et al.[9] relatam que uma hora de atraso na IOT aumenta 1,019 vezes a mortalidade em 28 dias. A esse respeito nossos dados são consistentes pois 48,4 % dos pacientes que falharam na VNI foram a óbito.

Em conclusão, pacientes infectados com COVID-19 que apresentam necessidade de suporte ventilatório mecânico, são em maioria homens, com pelo menos uma comorbidade e podem se beneficiar do uso da VNI, desde que a escolha seja feita de forma segura levando em consideração a gravidade da hipoxemia, pois, pacientes que apresentam níveis de PaO_2 abaixo de 60 mmHg tendem a falhar com esse suporte, atrasando a IOT e aumentando a mortalidade.

Devido a característica retrospectiva e observacional, este estudo apresenta limitações relacionadas ao registro de dados, impossibilitando a coleta de algumas informações e possibilitando viés de informação. O estudo foi realizado em somente uma UTI, o que gerou um número pequeno de participantes, portanto sugere-se mais estudos a respeito.

REFERÊNCIAS

1. Elsayed HH, Hassaballa AS, Ahmed TA, Gumaa M, Sharkawy HY, Moharram AA. Variation in outcome of invasive mechanical ventilation between different countries for patients with severe COVID-19: a systematic review and meta-analysis. *PLoS One*. 2021;16(6):e0252760.

2. Zeiser FA, Donida B, Costa CA, Ramos GO, Scherer JN, Barcellos NT, et al. First and second COVID-19 waves in Brazil: a cross-sectional study of patients' characteristics related to hospitalization and in-hospital mortality. *Lancet Reg Health Am*. 2022;6:100107.

3. Organização Pan-Americana da Saúde [Internet]. Histórico da pandemia de COVID-19. OPAS;2022 [acesso em 24 nov 2022]. Disponível em: https://www.paho.org/pt/covid19/historico-da-pandemia-covid-19.

4. Gujski M, Jankowski M, Rabczenko D, Goryński P, Juszczyk G. The prevalence of acute respiratory distress syndrome (ARDS) and outcomes in hospitalized patients with COVID-19 – a study based on data from the Polish National Hospital register. *Viruses*. 2022;14(1):76.

5. Fu Y, Guan L, Wu W, Yuan J, Zha S, Wen J, et al. Noninvasive ventilation in patients with COVID-19-related acute hypoxemic respiratory failure: a retrospective cohort study. *Front Med*. 2021;8:638201.

6. Thomas P, Baldwin C, Bissett B, Boden I, Gosselink R, Granger CL, et al. Physiotherapy management for COVID-19 in the acute hospital setting: clinical practice recommendations. *J Physiother*. 2020;66(2):73-82.

7. Organização Pan-Americana da Saúde. COVID-19 manejo clínico: orientação dinâmica. [Internet]: OPAS;2021 [acesso em 24 nov 2022]. Disponível em: https://iris.paho.org/bitstream/handle/10665.2/53296/OPASWBRAPHECOVID- 19210008_por.pdf?sequence=1&isAllowed=y.

8. Forrest IS, Jaladanki SK, Paranjpe I, Glicksberg BS, Nadkarni GN, Do R. Non-invasive ventilation versus mechanical ventilation in hypoxemic patients with COVID-19. *Infection*. 2021;49(5):989-97.

9. Bai L, Ding F, Xiong W, Shu W, Jiang L, Liu Y, et al. Early assessment of the efficacy of noninvasive ventilation tested by HACOR score to avoid delayed intubation in patients with moderate to severe ARDS. *Ther Adv Respir Dis*. 2022;16:17534666221081042.

10. British Thoracic Society Standards of Care Committee. Non-invasive ventilation in acute respiratory failure. *Thorax*. 2002;57:192-211.

11. Ferrer M, Esquinas A, Leon M, Gonzalez G, Alarcon A, Torres A. Noninvasive ventilation in severe hypoxemic respiratory failure: a randomized clinical trial. *Am J Respir Crit Care Med*. 2003;168(12):1438-44.

12. Thille AW, Contou D, Fragnoli C, Córdoba-Izquierdo A, Boissier F, Brun-Buisson C. Non- invasive ventilation for acute hypoxemic respiratory failure: intubation rate and risk factors. *Crit Care*. 2013;17(6):R269.

13. Weigert RM, Garcia GF, Muniz JCN, Francio F, Fontoura F, Forgiarini Junior LA. Utilização da ventilação mecânica não invasiva em pacientes internados na unidade de terapia intensiva adulto: sucesso, insucesso, motivo da VNI, tempo de internação, alta ou óbito. *Clin Biomed Res*. 2021;41(1):6-11.

14. Grieco DL, Menga LS, Raggi V, Bongiovanni F, Anzellotti GM, Tanzarella ES, et al. Physiological comparison of high-flow nasal cannula and helmet noninvasive ventilation in acute hypoxemic respiratory failure. *Am J Respir Crit Care Med*. 2020;201(3):303-12.

15. Wang D, Hu B, Hu C, Zhu F, Liu X, Zhang J, et al. Clinical characteristics of 138 hospitalized patients with 2019 novel coronavirus-infected pneumonia in Wuhan, China. *JAMA*. 2020;323(11):1061-9.

16. Zhao D, Yao F, Wang L, Zheng L, Gao Y, Ye J, et al. A comparative study on the clinical features of coronavirus 2019 (COVID-19) pneumonia with other pneumonias. *Clin Infect Dis*. 2020;71(15):756-61.

17. Garcia WPD, Aguirre-Bermeo H, Buehler PK, Alfaro-Farias M, Yuen B, David S, et al. Implications of early respiratory support strategies on disease progression in critical COVID-19: a matched subanalysis of the prospective RISC-19-ICU cohort. *Crit Care*. 2021;25:175.

18. Ranzani OT, Bastos LSL, Gelli JGM, Marchesi JF, Baião F, Hamacher S, et al. Characterisation of the first 250,000 hospital admissions for COVID-19 in Brazil: a retrospective analysis of nationwide data. *Lancet Respir Med*. 2021;9(4):407-18.

19. Governo do Estado do Rio Grande do Sul (BR). Plano estadual de vacinação contra covid-19 do Rio Grande do Sul: embasamento, operacionalização e avaliação. Porto Alegre: Governo do Estado do Rio Grande do Sul; 2021.

20. Bain W, Yang H, Shah FA, Suber T, Drohan C, Al-Yousif N, et al. COVID-19 versus non- COVID-19 acute respiratory distress syndrome: comparison of demographics, physiologic parameters, inflammatory biomarkers, and clinical outcomes. *Ann Am Thorac Soc*. 2021;18(7):1202-10.

21. Guan L, Zhou L, Le Grange JM, Zheng Z, Chen R. Non-invasive ventilation in the treatment of early hypoxemic respiratory failure caused by COVID-19: considering nasal CPAP as the first choice. *Crit Care*. 2020;24:333.

22. Chacko B, Thomas L, Sharma R, Yadav B, Jeyaseelan L, Arul AO, et al. Noninvasive ventilation in the management of respiratory failure due to COVID-19 infection: experience from a resource-limited setting. *Mayo Clin Proc*. 2022;97(1):31-45.

23. Bhutta BS, Alghoula F, Berim I. Hypoxia. *StatPearls* [Internet]. 2022 [atualizado em 9 ago 2022; citado em 24 nov 2022]. Disponível em: https://www.ncbi.nlm.nih.gov/books/NBK482316/

24. Shebl E, Mirabile VS, Sankari A, Burns B. Respiratory failure in adults. *StatPearls* [Internet]. 2022 [atualizado em 7 jul 2022; citado em 24 nov 2022]. Disponível em: https://www.ncbi.nlm.nih.gov/books/NBK526127/

25. Bellani G, Laffey JG, Pham T, Madotto F, Fan E, Brochard L, et al. Noninvasive ventilation of patients with acute respiratory distress syndrome. insights from the LUNG SAFE study. *Am J Respir Crit Care Med*. 2017;195(1):67-77.

26. Okano H, Sakuraya M, Masuyama T, Kimata S, Hokari S. Respiratory support strategy in adults with acute hypoxemic respiratory failure: a systematic review and network meta-analysis. *JA Clin Rep*. 2022;8(1):34.

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Artigo Original

Implementando CRISPR/Cas9 no laboratório: um guia de como editar células para criar modelos celulares

A comprehensive guide to generating cell models in the lab using CRISPR/Cas9

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RESUMO

Modelos celulares são muito utilizados na pesquisa experimental e, idealmente, constituem o primeiro nível de modelo de estudo. A utilização de modelos celulares permite, por exemplo, a primeira avaliação de tolerabilidade e de segurança de fármacos e tratamentos experimentais, além de permitir a redução do uso de animais de experimentação. Muito conhecimento já foi gerado cultivando células de pacientes com patologias diversas – de doenças genéticas hereditárias a câncer. Entretanto, muitos pesquisadores não têm acesso às células de pacientes. O desenvolvimento da técnica de edição de genes CRISPR/Cas9, na última década, tornou a geração de modelos celulares muito mais acessível para pesquisadores do mundo todo, por permitir a edição de genes de interesse em múltiplos tipos celulares, incluindo células imortalizadas e de fácil cultivo. Nesse contexto, este artigo descreve como a edição gênica funciona e como utilizar essa ferramenta *in vitro* para produzir modelos celulares para estudo. Nosso objetivo é apresentar essa metodologia a laboratórios que tenham interesse em implementar edição genômica em seus projetos de pesquisa.

Palavras-chave: *Edição gênica, edição genômica, CRISPR/Cas9, edição de DNA, modelos celulares, linhagens celulares, knockout, engenharia genética, geração de nocaute, transfecção.*

ABSTRACT

Cellular models are extensively utilized in experimental research and serve as the primary level of study. These models enable the initial assessment of drug and treatment tolerability and safety, while also reducing the need for experimental animals. Significant knowledge has been acquired by cultivating cells from patients with various pathologies, ranging from hereditary genetic disorders to cancer. However, access to such cells is limited for many researchers. The advent of the CRISPR/Cas9 gene editing technique in the past decade has revolutionized the generation of cellular models, making it more accessible to researchers worldwide. This technique allows for the targeted editing of genes of interest in multiple cell types, including immortalized cells. This manuscript aims to provide a comprehensive overview of gene editing, while also being a guide for conducting initial experiments, with a primary focus on the development of cell models. Our primary goal is to introduce these techniques to labs in Brazil that are not familiar with them.

Keywords: *Gene editing, genome editing, CRISPR/Cas9, DNA editing, cell models, cell lines, knockout, genetic engineering, knockout models, transfection.*

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PARTE 1: SOBRE O CRISPR/CAS9

CRISPR/Cas9

A edição genômica por CRISPR/Cas9 revolucionou a pesquisa biotecnológica, pois permite que qualquer laboratório no mundo modifique genes com mais facilidade e menor custo do que as alternativas anteriormente disponíveis. A ferramenta utiliza apenas dois componentes: uma nuclease Cas9, que cliva a fita dupla de DNA, e o RNA guia (gRNA), que é complementar ao local de interesse no genoma e direciona a Cas9 ao sítio de edição. Uma vez no local específico, o complexo gRNA-Cas9 cliva a fita dupla de DNA. As quebras geradas nas duas fitas são altamente danosas para a célula, que se encarrega de reparar o dano imediatamente. Esse reparo pode ocorrer por dois processos principais: junção terminal não-homóloga (do inglês *non-homologous end joining,* NHEJ) e reparo direcionado por homologia (*homology-directed repair,* HDR). O NHEJ pode induzir pequenas mutações no local da clivagem quando a demanda por reparo é alta; já o HDR utiliza uma sequência homóloga como molde para realizar o reparo. Em células em divisão, quando o HDR é mais ativo, o molde utilizado é a cromátide-irmã. Nos protocolos de edição, a sequência de DNA que será utilizada como molde é fornecida à célula (geralmente introduzida junto com a Cas9 e o gRNA) e contém a alteração de interesse flanqueada por sequências homólogas ao ponto de edição no genoma. Ou seja, para induzir mutações aleatórias, só são necessários dois componentes (gRNA e Cas9); para editar sequências com precisão, são necessários três (gRNA, Cas9 e sequência molde com homologia)[1]. Mais detalhes sobre a origem do CRISPR/Cas9, sua estrutura e suas aplicações podem ser encontrados em diversos artigos de revisão na literatura[1-4].

O RNA guia

Estrutura da molécula para edição genômica

De modo simplificado, o gRNA desenvolvido para edição genômica possui duas partes: um esqueleto (ou *scaffold*) de sequência fixa, que é responsável por recrutar a nuclease e por estabilizar o complexo, e uma sequência espaçadora (ou *spacer),* composta de 20 nucleotídeos (nt) variáveis, que é responsável por reconhecer o alvo e direcionar a nuclease ao ponto de clivagem. A sequência espaçadora ainda é dividida entre *seed* e *non-seed.* A primeira tem de 10 – 12 nt e corresponde à porção mais próxima à extremidade 3'; essa porção é crítica ao reconhecimento do alvo e à atividade do sistema, requerendo completa complementariedade entre a sequência e o alvo. A região *non-seed*, por outro lado, localiza-se na extremidade 5' do gRNA e permite alguns desemparelhamentos (*mismatches*) sem impacto considerável na edição[1]. A Figura 1 ilustra as características do gRNA.

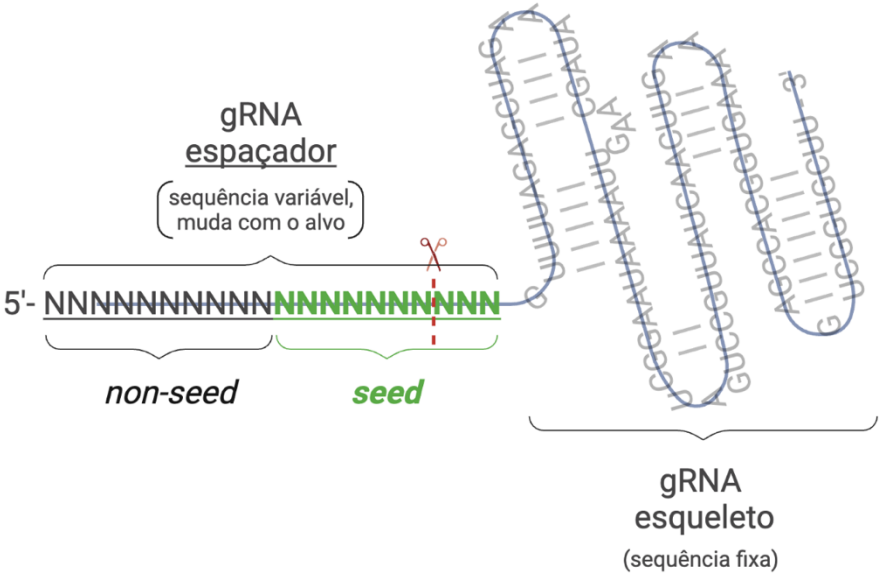

Figura 1: Estrutura do RNA guia (gRNA) utilizado para edição genômica no sistema CRISPR/Cas9.

Nota: O gRNA é uma molécula de RNA com aproximadamente 100 nucleotídeos, composta por uma sequência fixa chamada de esqueleto (ou *scaffold*, em inglês) e uma sequência de 20 nucleotídeos que é complementar ao local do genoma a ser editado, chamada de espaçador (ou *spacer*, em inglês; sublinhado na figura). Esta sequência espaçadora, portanto, é variável e é desenhada de acordo com o experimento. Ela ainda é subdivida em *seed* (em verde) e *non-seed* (em preto).

Fonte: Criado com BioRender.com.

Desenho do gRNA

O desenho do gRNA também deve levar em consideração o objetivo do projeto – a edição é para nocautear um gene (independente da alteração induzida), ou é para modificar sequências com precisão (como inserir genes ou induzir mutações específicas)? No primeiro caso, a sequência do gRNA será mais importante do que a sua localização, porque buscamos o guia mais eficiente que tenha como alvo o gene de interesse (independentemente de o guia reconhecer o éxon 1 ou o éxon 5, por exemplo). Por outro lado, para induzir alterações específicas, o local onde o gRNA anela no genoma é fundamental e deve ser priorizada. Isso se deve à necessidade de induzir a clivagem do genoma o mais próximo o possível do local da edição. Se a distância entre o ponto de clivagem e a mutação a ser introduzida for de 10 nt, a eficiência de edição cai para aproximadamente a metade dos alelos e para apenas 20% de edições bialélicas, em comparação a 1 nt de distância; se a distância da clivagem for de 30 nt do ponto da mutação, a eficiência de edições bialélicas é praticamente nula. Entretanto, se o objetivo for introduzir alterações em heterozigose, o ponto de clivagem idealmente deve ser entre 5 a 20 nt distante da mutação[5,6].

Para nocautear genes, deve-se evitar as sequências que codificam o início e o final da proteína, pois códons de iniciação e terminação alternativos poderão ser utilizados e interferir no produto. Além disso, uma opção para aumentar a eficiência do sistema é utilizar múltiplos gRNAs próximos, que induzirão a deleção da sequência entre os seus pontos de clivagem[7].

Ferramentas para desenhar o gRNA

Existem múltiplas ferramentas online para o desenho do gRNA, conforme a Tabela 1 demonstra. Em geral, são ferramentas simples, em que o usuário escolhe o genoma da espécie (humano, camundongo, etc.), o gene de interesse e a modificação desejada. Para selecionar o gene, é possível indicar o número da sequência de referência do mesmo, ou as inserir manualmente. Caso a última opção seja escolhida, deve-se inserir a sequência dos éxons e não a sequência genômica (porque pode desenhar guias em íntrons) e tampouco o cDNA (porque pode desenhar guias nas junções éxon-éxon, que serão inativos no genoma).

Tabela 1: Exemplos de ferramentas online para desenhar o gRNA.

Ferramenta	Website
ATUM CRISPR gRNA Design tool	https://www.atum.bio/eCommerce/cas9/input
Benchling CRISPR Guide RNA Design	https://www.benchling.com/crispr/
Cas-OFFinder	http://www.rgenome.net/cas-offinder/
CHOP-CHOP	https://chopchop.cbu.uib.no/
CRISPick	https://portals.broadinstitute.org/gppx/crispick/public
CRISPOR	http://crispor.tefor.net/
CRISPR-ERA	http://crispr-era.stanford.edu/
E-CRISP	http://www.e-crisp.org/E-CRISP/designcrispr.html
GenScript CRISPR sgRNA Design Tool	https://www.genscript.com/gRNA-design-tool.html
IDT CRISPR-Cas9 guide RNA	https://www.idtdna.com/site/order/designtool/index/CRISPR_CUSTOM
Invitrogen TrueDesign Genome Editor	https://www.thermofisher.com/us/en/home/life-science/genome-editing/invitrogen-truedesign-genome-editor.html
Off-Spotter	https://cm.jefferson.edu/Off-Spotter/
Synthego CRISPR Design Tool	https://www.synthego.com/products/bioinformatics/crispr-design-tool
CRISPRDB	https://crisprdb.org/

Independente da ferramenta utilizada, é sempre recomendado escolher a sequência com maior escore no alvo e menor número de *off-targets*, ou seja, de possível atividade fora do alvo. Também é interessante utilizar mais de uma ferramenta, pois frequentemente os programas de predição apresentam resultados discordantes. Entretanto, a eficiência predita por esses programas, mesmo quando concordantes, nem sempre corrobora o que é visto experimentalmente[8].

Eficiência

A eficiência de um guia vai depender não só da sua sequência intrínseca, mas também da estrutura secundária do alvo no genoma, que inclui o estado da cromatina, a presença de proteínas associadas, etc.[8]. Sendo assim, cada guia é único e terá atividade diferente, podendo variar consideravelmente mesmo que sejam sequências adjacentes. Por isso, sempre que possível, é indicado que se teste mais de um gRNA para cada alvo de interesse. Guias ineficientes tornarão todo o processo mais dispendioso.

Na Figura 2, apresentamos dois gRNAs cuja atividade foi determinada experimentalmente. Observe como a eficiência dos guias (estimada a partir da quantidade de inserções e deleções (*indels)* geradas) varia (Figura 2A), mesmo ligando no alvo a apenas 90 pb distantes entre si (Figura 2B).

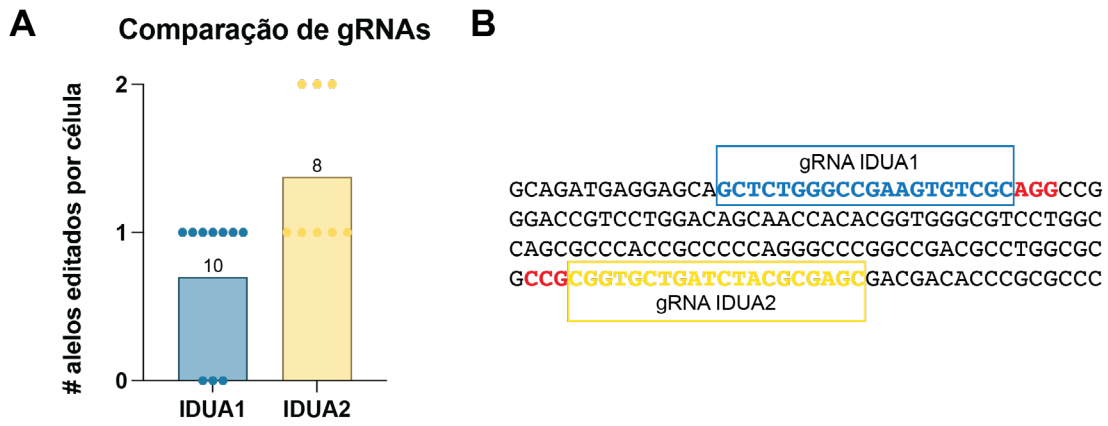

Figura 2: Comparação da eficiência de indução de mutações com guias diferentes.

A: No gene *IDUA*, o guia IDUA1 gerou *indels* em apenas 35% dos alelos analisados (20 no total) e não produziu nenhuma célula com alteração bialélica; já o guia IDUA2 gerou *indels* em 70% dos alelos, sendo que nenhum clone permaneceu sem *indels* e três tinham edição bialélica. Para este experimento, células HEK293 foram submetidas à edição e isoladas para obtenção de clones únicos. Cada ponto representa um clone e o número acima das barras representa o número de clones analisados para cada condição. Dados determinados experimentalmente; B: Localização dos guias no gene IDUA, que estão somente 90 pb distantes entre si. A sequência PAM está representada em vermelho.

Quantificando as indels para verificar a eficiência do sistema

A quantidade de *indels* introduzidas no alvo é uma medida indireta da eficiência de clivagem do sistema. Quanto mais o sistema induzir a quebra no alvo, maior é a chance de o reparo por NHEJ introduzir alterações na sequência. Assim, para sabermos a atividade de um gRNA, podemos simplesmente quantificar a frequência de *inde*ls presentes na população de células submetidas à edição, sem a necessidade de isolar clones e sequenciá-los individualmente. Guias eficientes não só introduzirão *indels* mais facilmente, como também permitirão uma edição precisa utilizando sequências doadoras para o reparo direcionado por recombinação. Portanto, quanto mais clivagens ocorrerem, mais edição ocorrerá, independente do mecanismo.

Testar diferentes guias para o mesmo alvo e avaliar a eficiência de cada um pode ser uma boa estratégia. Para isso, basta editar grupos de células separadamente com cada um dos guias, aguardar 48 a 72 h (para dar tempo suficiente de a edição ocorrer), extrair e sequenciar o DNA de parte das células, e quantificar as *indels* presentes no alvo, que é feita por programas de análise de decomposição de sequências (Figura 3A).

Para fazer essa análise, primeiro é necessário desenhar primers para amplificar a região de interesse. Os programas de análise recomendam, em geral, amplicons de 700 pb que contenham a região da edição, aproximadamente, no meio do amplicon. Assim, no sequenciamento, haverá uma região à montante da edição que terá a mesma sequência em todas as moléculas de DNA e apresentará uma aparência "limpa" no cromatograma, e uma porção após o ponto da edição que conterá sequências diversas decorrentes da edição, com uma aparência "bagunçada" (Figura 3B).

Essas ferramentas, como o TIDE (https://tide.nki.nl) ou o ICE (https://ice.synthego.com) comparam a quantidade de sequências diferentes e a proporção delas em relação à sequência selvagem, de acordo com o tamanho e a posição dos picos no cromatograma. Por isso, é importante que se tenha sequenciamentos com picos únicos e com, pelo menos, 200 pares de base inalterados antes do ponto da edição. Esses programas também nos fornecem a porcentagem de *indels* detectada (Figura 3C-D) e podemos utilizar esse número como a medida indireta de clivagem de um guia. Assim, ao testarmos vários guias, escolhemos aquele que apresenta a maior geração de *indels* (e, portanto, maior clivagem).

A ferramenta ICE (ice.synthego.com) também fornece um escore de nocaute (*KO score*), que informa qual proporção das *indels* geradas são potencialmente nocauteadoras, por serem inserções ou deleções de nucleotídeos não múltiplos de 3 e, portanto, alterações fora de fase (ou *frameshift*).

No exemplo I (Figura 3B), a sequência antes do local da quebra (sinalizado pela linha pontilhada) está bem clara, com picos únicos. Após o ponto da quebra, vários picos surgem, de tamanhos variados e com posições apresentando até as 4 bases. Isso acontece porque existem múltiplas *indels* ocasionadas e cada sequência vai apresentar um perfil de picos distinto. Por outro lado, no exemplo II, observamos apenas duas sequências distintas após o ponto de edição. Isso porque uma mesma mutação (duplicação de 1 A) predominou nos eventos de *indels* gerados.

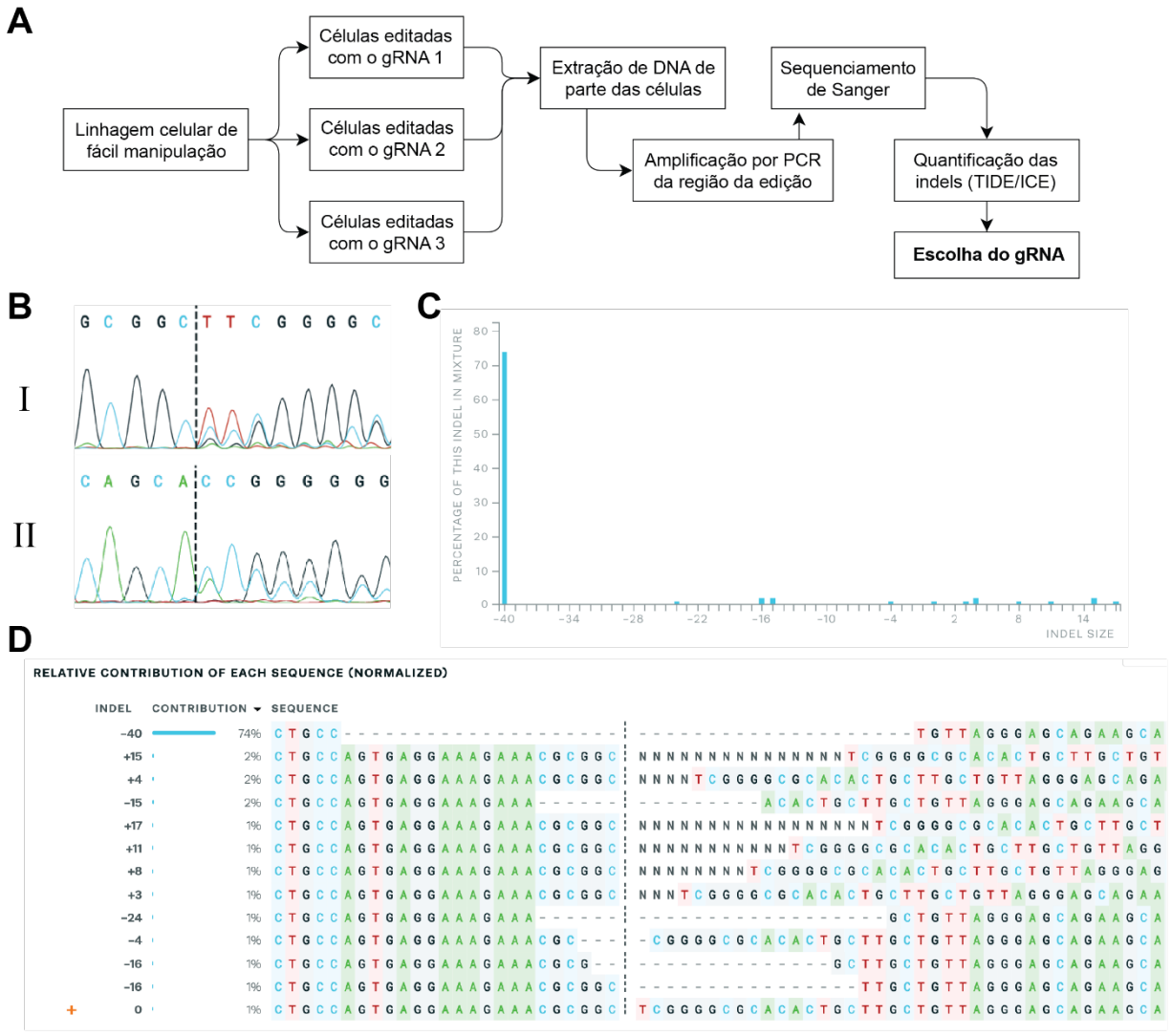

Figura 3: Analisando a eficiência de clivagem do gRNA.

A: Etapas para escolher o gRNA mais eficiente (ou seja, o que induz mais clivagens e, consequentemente, *indels*, no alvo); B: Cromatogramas gerados pelo sequenciamento de Sanger, por meio dos quais é possível observar a presença de múltiplas sequências (evidenciadas por sobreposição de picos) após o ponto de clivagem previsto para cada gRNA (representado pela linha pontilhada); C: Geralmente, há predomínio de uma indel, mas muitas sequências diferentes podem ser produzidas; D: A proporção de sequências alteradas em relação à sequência selvagem (ou seja, a porcentagem de *indels* na população) corresponde à medida indireta de eficiência do gRNA.
C e D representam exemplos de resultados de uma análise feita na ferramenta ICE, que mostra o perfil e a frequência (ou contribuição) de todas *indels* identificadas na população analisada.

Essa análise pode ser usada não somente para determinar a eficiência da clivagem do gRNA, mas também para caracterizar a população editada e para tomada de decisões no fluxo de geração do modelo. Considere hipoteticamente que uma população de células foi submetida à edição no dia 0. No dia 2, uma parte das células foi coletada e a análise de decomposição de *indels* foi feita, apresentando 20% de *indels*. As células foram mantidas em cultivo e, no dia 10, a análise de decomposição foi refeita, acusando uma proporção de *indels* de 70%. Nesse caso, a edição pode estar conferindo uma vantagem seletiva para essas células, que estão dominando a população. Se o objetivo for obter uma população editada (independente do genótipo), basta manter essas células em cultivo que, eventualmente, todas as células serão editadas/nocautes. Por outro lado, o contrário também poderá acontecer – se o gene for fundamental para a sobrevivência ou proliferação celular e for nocauteado pelas *indels* geradas, as células editadas podem estar morrendo, ou podem estar proliferando mais lentamente que

células não editadas, e serão eventualmente perdidas, caso sejam mantidas em cultivo.

Em casos em que o melhor gRNA já foi escolhido e, mesmo assim, a eficiência da edição é pequena, pode-se fazer múltiplos ciclos de edição para aumentar a proporção de células editadas antes de realizar o isolamento e a expansão clonal, aumentando a chance de se obter um clone editado. Entre os ciclos, é interessante fazer a análise de decomposição para avaliar o incremento de edição a cada ciclo e se há edição o suficiente para fazer o isolamento clonal, caso esta etapa esteja no escopo do projeto.

Entretanto, vale ressaltar que a análise das sequências da população mista pode superestimar o número de clones editados, isolados e viáveis. Usher e colaboradores[9] obtiveram apenas 1 % de clones editados por recombinação direcionada por homologia, embora a previsão pela análise da população mista era de 12 %.

Cas9 e a sequência PAM

A sequência PAM (do inglês *protospacer adjacent motif,* ou motivo adjacente ao protoespaçador) é uma sequência de reconhecimento do complexo gRNA-Cas9. O complexo rastreia o genoma em busca desta sequência e, a cada PAM encontrada, ele interroga a sequência adjacente em busca de complementariedade com o gRNA. Quando a sequência complementar é encontrada, a Cas9 dissocia as fitas de DNA no genoma e há a invasão do gRNA para formar um dúplex DNA-RNA (Figura 4). Com a complementariedade perfeita, a Cas9 cliva a dupla fita de DNA 3 pb à montante da PAM. Ou seja, a sequência PAM é encontrada no genoma, no alvo, e não no complexo CRISPR-Cas9. Portanto, ao desenhar o gRNA, os 20 nt não devem incluir a PAM[1].

A PAM varia de acordo com a Cas e com a espécie. A Cas mais utilizada é a Cas9 de *Streptococcus pyogenes* (SpyCas9 ou SpCas9) e a sequência PAM reconhecida por ela é "NGG". Consequentemente, só é possível realizar uma edição por CRISPR/Cas9 usando SpCas9 em locais onde há uma sequência NGG adjacente. Na ausência de NGG no alvo, é possível utilizar outros sistemas, uma vez que outras Cas reconhecem sequências PAM diferentes (Tabela 2).

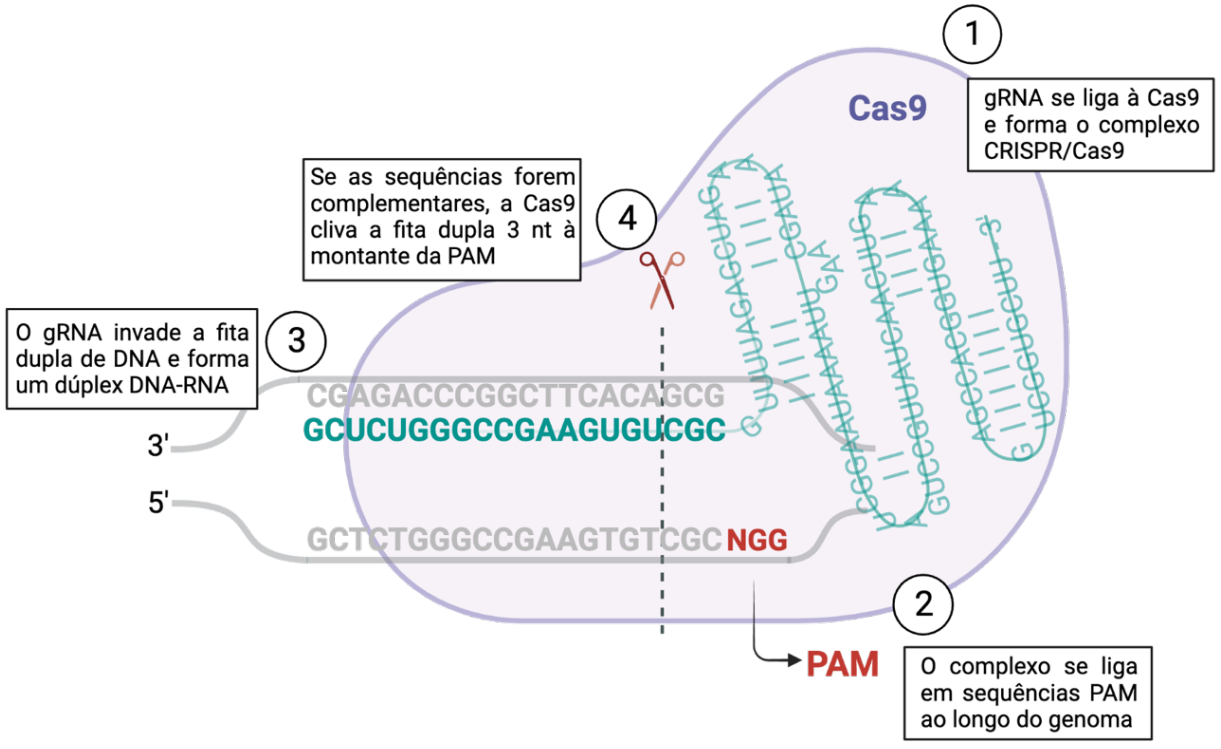

Figura 4: Estrutura e ação do complexo CRISPR/Cas9.

1: O esqueleto do gRNA se liga à Cas9, formando um complexo; 2: Esse complexo percorre o genoma e, a cada sequência PAM (em vermelho) encontrada, ele se liga ao DNA, invadindo a fita dupla; 3: A partir daí, busca o pareamento entre sua sequência no gRNA e a sequência no genoma. Se a sequência não for complementar, o complexo se dissocia do genoma e busca um novo alvo; 4: Se a sequência for complementar, o complexo se estabiliza e a Cas9 cliva a fita dupla de DNA à montante da sequência PAM.

Fonte: Criado com BioRender.com.

Tabela 2: Tipos de Cas mais utilizadas em protocolos de edição e suas diferentes sequências PAM.

Nuclease	Organismo de origem	Sequência PAM (5'- 3')
SpCas9	*Streptococcus pyogenes*	NGG
SaCas9	*Staphylococcus aureus*	NGRRT or NGRRN
NmeCas9	*Neisseria meningitidis*	NNNNGATT
CjCas9	*Campylobacter jejuni*	NNNNRYAC
StCas9	*Streptococcus thermophilus*	NNAGAAW
LbCpf1 (Cas12a)	*Lachnospiraceae* bacterium	TTTV
AsCpf1 (Cas12a)	*Acidaminococcus* sp.	TTTV
AacCas12b	*Alicyclobacillus acidiphilus*	TTN
BhCas12b v4	*Bacillus hisashii*	ATTN, TTTN e GTTN
Cas14	*Uncultivated archea*	Sequências ricas em T, ex. TTTA
Cas3	análise *in silico* de vários genomas de procariotos	Não necessita de PAM

Fonte: synthego.com

DNA molde ou sequência homóloga

Para geração de *knock-ins* (termo em inglês que se refere à inserção de sequências em um genoma), é necessário fornecer à célula uma molécula de DNA contendo a alteração de interesse, que chamamos de sequência doadora ou sequência molde. Essa sequência molde pode ter tamanho variável – que pode inserir de um a milhares de pares de bases – e deve conter sequências com homologia ao local de edição no genoma, os chamados *braços homólogos*. Esses braços homólogos têm a função de anelar ao sítio de edição e servir como molde para que o sistema de reparo celular o utilize como modelo para reparar a fita clivada (Figura 5). As possibilidades de edição utilizando esse mecanismo são múltiplas, e incluem inserir genes inteiros, promover excisão de éxons específicos, deletar sequências e corrigir mutações de ponto, por exemplo[10].

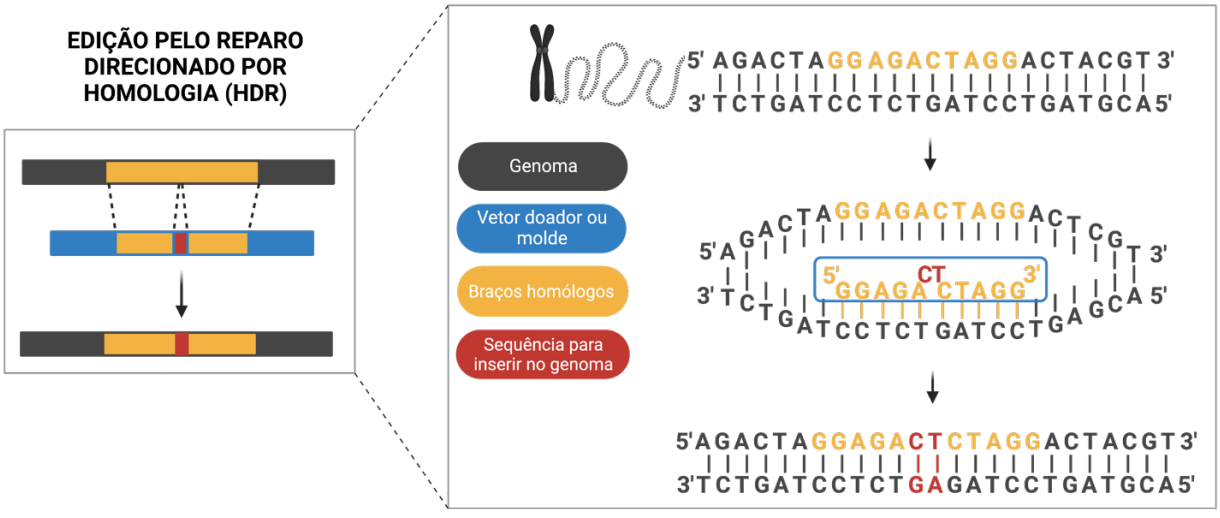

Figura 5: Inserção precisa de sequências de DNA no genoma pelo reparo direcionado por homologia (HDR).

Nota: Para que o reparo direcionado por homologia ocorra, como o nome já diz, é necessária a presença de uma sequência homóloga ao local de edição no genoma, isto é, sequências complementares que vão se anelar ao genoma. Após a indução da quebra da fita dupla de DNA pelo CRISPR/Cas9, a célula busca sequências homólogas para usar como modelo e reparar o DNA. Quando sequências extras são propositalmente inseridas entre os braços homólogos, é possível "enganar" o sistema de reparo e fazer com que ele inclua essas sequências no genoma, cimentando a modificação.

Fonte: Criado com BioRender.com.

O tamanho da sequência a ser introduzida no genoma é o maior determinante no momento de desenhar o molde e escolher o vetor de entrega, uma vez que alguns vetores têm limite de carga. O tamanho dessa sequência molde também vai interferir na eficiência do processo.

No caso de mutações de ponto, a sequência doadora deve ser completamente homóloga ao genoma, exceto na base correspondente à mutação de interesse. Nesses casos de edição de poucos nucleotídeos, é recomendado utilizar oligonucleotídeos de fita simples (ssODN) de até 100 nt de comprimento,

contendo aproximadamente 40 nt de homologia em cada extremidade[11]. Para inserir sequências maiores, de centenas de nucleotídeos, podem ser utilizados plasmídeos e vetores virais. O tamanho dos braços homólogos nesses casos ainda é controverso, mas estima-se que cada braço deva ter, em geral, 50 % do tamanho da sequência a ser introduzida no genoma, ou no mínimo 500 pb para sequências acima de 1000 pb. Embora a utilização de braços homólogos menores que 500 pb seja possível, a eficiência do processo pode diminuir[12]. Em resumo, recomenda-se braços homólogos de cerca de 50 pb para edições pequenas (< 10 pb) e em torno de 200 a 500 pb para inserção de sequências grandes, de centenas de bases (como inserção de genes, por exemplo). Em desenhos experimentais simples, alguns dos programas para desenho de gRNA mencionados na Tabela 1 também fornecem sugestões de sequências molde para recombinação.

Outra questão muito importante a ser considerada é que, por conter a sequência praticamente idêntica ao genoma e ao gRNA, a sequência doadora pode ser clivada pelo complexo CRISPR/Cas9. Inclusive, uma vez que a edição tenha ocorrido corretamente no genoma, o CRISPR poderá clivar novamente a fita já editada, que poderá ser reparada por NHEJ e gerar *indels* no local. Portanto, para evitar tanto a clivagem do vetor doador quanto a clivagem dos alelos já eficientemente editados, é interessante inserir mutações (silenciosas para sequências codificantes) na sequência doadora (Figura 6). A maneira mais significativa de aumentar a eficiência de *knock-in* é inserindo mutações silenciosas 1) na sequência PAM, 2) o mais próximo da PAM o possível e 3) inserindo mais de uma alteração[13].

Considere o exemplo da Figura 6. O objetivo é substituir um T por um C no códon destacado (cTg > cCg), gerando uma mutação de ponto. Esse códon contém parte da sequência PAM (TGG). Caso a edição ocorra eficientemente, a nova sequência continuará contendo uma sequência PAM (CGG) e a sequência do gRNA não será alterada. Nessa situação, o complexo gRNA-Cas9 continuará se ligando neste mesmo local e continuará clivando a mesma fita, mesmo que ela já tenha sido editada, até que eventualmente *indels* serão inseridas no local, impedindo o complexo de agir novamente. Assim, o produto final será composto majoritariamente por sequências com *indels* e praticamente nenhuma sequência com a edição de interesse. Para contornar este problema, podemos inserir algumas alterações silenciosas na sequência molde, de modo que o complexo gRNA-Cas9 não consiga mais reconhecer a sequência já editada e acabe perdendo sua atividade no local. No exemplo, duas bases foram substituídas (C > T) no genoma, em dois códons diferentes, sem alterar a sequência de aminoácidos da proteína.

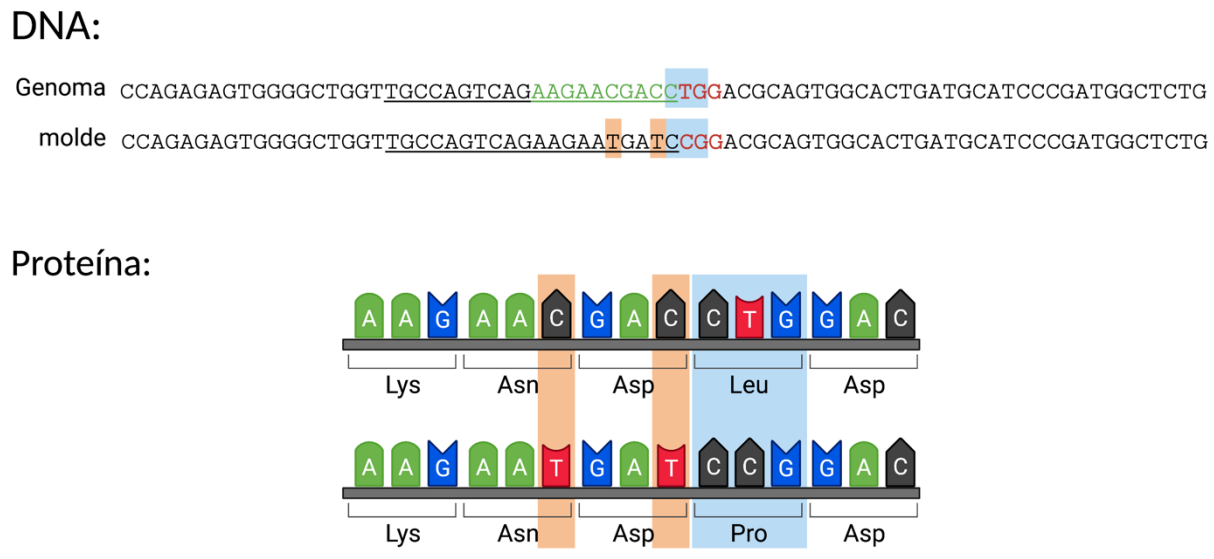

Figura 6: Desenho de sequência molde para reparo direcionado por homologia (HDR).

Nota: O códon para ser editado (destacado em azul) contém parte da sequência PAM (em vermelho). Com a edição correta do nucleotídeo de interesse (T>C), a sequência PAM permanece ativa (tGG>cGG) e o sistema continuaria clivando a mesma fita de DNA, mesmo após o evento de reparo e recombinação. Adicionando alterações silenciosas (destacadas em laranja) na porção *seed* do gRNA (gRNA sublinhado, porção *seed* em verde), garantimos que o sistema não clive o DNA já editado, uma vez que ele não reconhecerá mais essa sequência. A proteína produzida, por sua vez, terá alteração de aminoácidos somente no códon de interesse (em azul, Leu > Pro) e não nas alterações silenciosas (Asn > Asn e Asp > Asp).

Fonte: Criado com BioRender.com.

Antes de desenhar a sequência doadora (e o gRNA, inclusive), é recomendado sequenciar o alvo na célula de interesse. A presença de polimorfismos pode afetar significativamente a eficiência de clivagem ou de recombinação, pois a presença de um único nucleotídeo alterado pode inibir por completo o reparo por recombinação[14].

Entregando os componentes do complexo CRISPR/Cas9

A maneira mais economicamente acessível de entrega dos componentes do sistema CRISPR/Cas9 às células é por meio de plasmídeos que, em um único vetor, expressam o gRNA e a Cas9. Existem diversos plasmídeos disponíveis que, além do CRISPR, também expressam proteínas fluorescentes ou genes de resistência a antibióticos, facilitando a seleção das células transfectadas e potencialmente editadas. Estes plasmídeos são uma boa opção de baixo custo para edição de linhagens celulares de fácil manipulação. As desvantagens do uso desse sistema são a baixa eficiência de transfecção e de edição de células primárias[15] e o aumento da atividade de clivagem fora do alvo (os *off-targets*), uma vez que o plasmídeo permanece ativo por dias dentro das células[16]. Além disso, é necessário ter conhecimento básico em clonagem para poder inserir as sequências de gRNA desejadas (Figura 7A).

A alternativa aos plasmídeos mais utilizada é a entrega dos componentes já nas suas formas ativas, pré-complexando a molécula de gRNA à proteína Cas9 *in vitro*, formando um complexo ribonucleoproteico (RNP) que pode ser transfectado às células. Por não precisar ser transcrito ou traduzido, o complexo RNP tem ação imediata após ser internalizado, resultando em edição mais rápida e muito mais eficiente (Figura 7B). Outra vantagem é o menor tempo de permanência nas células, minimizando a ação fora do alvo[16].

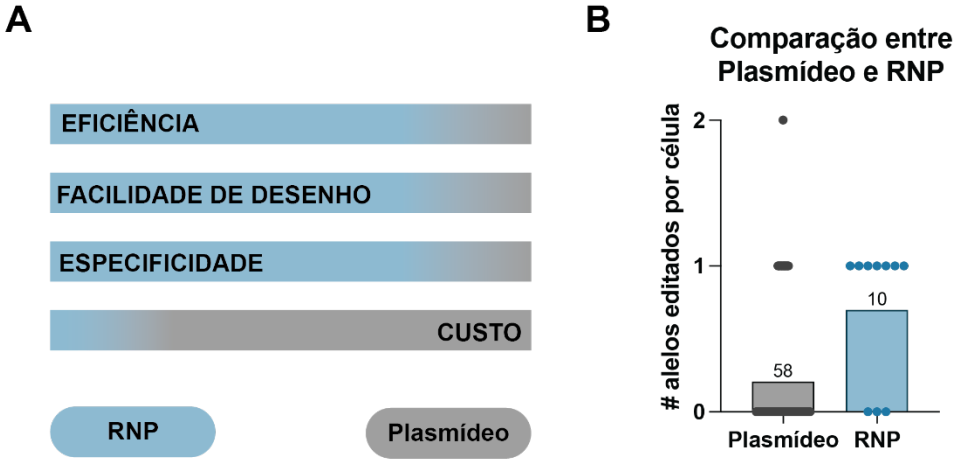

Figura 7: Comparação entre plasmídeo e complexo ribonucleoproteico (RNP) para edição por CRISPR/Cas9.

A: O uso de plasmídeos, embora tenha menor custo, é menos eficiente, tem mais atividade inespecífica e demanda maior conhecimento de clonagem para produção do vetor. Nesse contexto, uso de RNP é mais simples e mais eficiente; B: Comparação entre as duas formas de entrega. Ambas as condições (plasmídeo ou RNP) foram avaliadas utilizando a mesma sequência de gRNA (IDUA1, apresentado na Figura 2) com alvo no gene *IDUA*. Na condição Plasmídeo, apenas 12 alelos foram editados dos 116 sequenciados, resultando numa eficiência de edição de apenas 10%. Na condição RNP, *indels* foram encontradas em 7 dos 20 alelos analisados, o que indica eficiência de 35%. No gráfico, cada ponto representa um clone e o número acima das barras representa o número de clones analisados para cada condição.

Uma terceira opção de entrega é a utilização da Cas9 na forma de mRNA, atuando como um intermediário entre a entrega da proteína pronta e da entrega como DNA no plasmídeo. Como mRNA, a Cas9 é produzida mais rapidamente do que como plasmídeo, pois pula a etapa de transcrição de DNA em RNA e passa diretamente à tradução para proteína. Estima-se que a tradução da Cas9 nas células eucarióticas leve apenas 5 minutos[17]. Além de iniciar a edição rapidamente, o mRNA não permanece nas células por muito tempo, diminuindo a chance de off-targets[16].

Embora o uso de mRNA seja mais eficiente que a utilização de plasmídeos, algumas considerações devem ser observadas ao se utilizar Cas9 mRNA, principalmente em células primárias. Por exemplo, foi demonstrado que inserir mRNA de Cas9 em células-tronco hematopoiéticas pode causar uma maior repressão transcricional global em comparação com a Cas9 proteína[18]. Também é interessante observar que, embora a molécula do mRNA contenha menos nucleotídeos que a do plasmídeo, o tamanho da molécula de mRNA é maior, uma vez que o mRNA

é linearizado enquanto o plasmídeo é altamente compactado, o que pode interferir na transfecção[17].

Off-targets – a edição fora do alvo

A atividade fora do alvo (ou *off-target*, em inglês) é a atividade inespecífica do sistema. Sempre que possível, é recomendado desenhar o experimento de modo que se tenha o mínimo possível de atividade inespecífica. Guias com alta atividade fora do alvo tendem a ter atividade no alvo reduzida[19]. Além disso, caso ocorram mutações em outros sítios, existe a possibilidade de o fenótipo observado na célula ser um efeito das alterações inespecíficas e não efeito da edição no gene alvo.

A maioria dos programas de desenho de gRNA já fornece os possíveis sítios inespecíficos e a probabilidade de atividade do sistema nesses pontos. Algumas ferramentas específicas, como o COSMID (https://crispr.bme.gatech.edu) e o Cas-OFFinder (http://www.rgenome.net/cas-offinder) fornecem uma lista extensa de possíveis sítios inespecíficos para cada gRNA. Frequentemente, não é possível desenhar gRNAs no local de interesse sem que haja sítios inespecíficos possíveis; nesses casos, é preferível escolher gRNAs que tenham esses *off-targets* em regiões intergênicas ou intrônicas, evitando as regiões codificantes. A análise extensa de *off-targets* se torna mandatória em casos de utilização do CRISPR/Cas9 para terapia, e diversas técnicas já foram descritas[20].

Uma alternativa para evitar a ação inespecífica do CRISPR/Cas9 é utilizar enzimas Cas9 de alta fidelidade (*high fidelity* Cas9, ou Hifi Cas9)[21]. Elas apresentam modificações em sua estrutura, que as tornam menos propensas a atuar na presença de bases desemparelhadas.

PARTE 2: SOBRE AS CÉLULAS

Escolhendo a linhagem celular

A escolha do tipo celular é um ponto muito importante na geração de um modelo celular e depende do objetivo e da disponibilidade dos recursos. Células primárias possuem tempo de cultivo limitado e, por isso, impõem uma restrição importante no modelo, o que deve ser considerado cuidadosamente antes de iniciar o projeto. A utilização de células imortalizadas é mais fácil, uma vez que essas linhagens crescem facilmente e podem ser cultivadas indefinidamente[22].

Ao planejar o projeto, deve-se considerar se o tipo celular é importante para o estudo e se a análise a ser feita no modelo depende ou não do tipo celular. Muitas perguntas de pesquisa podem ser respondidas com modelos celulares genéricos, sem a necessidade de se utilizar um tipo celular específico.

Exemplos de projetos que independem do tipo celular:

- Avaliar a morte celular causada por um novo fármaco em células que não expressem o gene A.
- Utilizar células nocaute para o gene B como controle negativo em um ensaio.
- Avaliar se a ausência de expressão do gene C aumenta a proliferação celular.

Entretanto, muitas vezes, busca-se utilizar modelos mais relevantes para o objeto de pesquisa, gerando modelos com tipos celulares mais informativos.

Exemplos:

- Avaliar o acúmulo de metabólitos em células nocautes para o gene A, o qual está relacionado a uma doença neurodegenerativa – pode ser mais interessante utilizar células neurais.
- Avaliar a contratilidade de células musculares após o tratamento com um novo fármaco, como potencial terapia para uma miopatia – outros tipos celulares não terão função contrátil.
- Avaliar o efeito do gene B (que só é expresso em fibroblastos) na regulação da proteína C – como só há expressão de B em fibroblastos, nocautear esse gene em outros tipos celulares não surtirá efeito na proteína C.

Além da relevância biológica do tipo celular, também pode-se considerar a facilidade de manipulação das células a cada etapa de edição, como o número de cromossomos (algumas linhagens são aneuploides, com múltiplos cromossomos, aumentando o número de alelos a serem modificados), a transfecção (alguns tipos celulares não toleram bem o processo de transfecção) e o isolamento de clones (algumas linhagens não expandem bem quando estão em baixa densidade celular).

Métodos de transfecção e a variação entre os tipos celulares

Transfecção é o nome dado ao processo de introduzir ácidos nucleicos em células eucarióticas utilizando métodos não-virais (para métodos virais, utiliza-se o termo transdução). Os métodos de transfecção são divididos em físicos e químicos, sendo os mais frequentemente utilizados a eletroporação (físico) e a utilização de moléculas catiônicas, que incluem lipossomos e polímeros (químico)[22].

A eletroporação consiste em aplicar um campo elétrico numa solução de células, gerando poros na membrana plasmática que permitem a entrada de moléculas. A transfecção química, por outro lado, utiliza-se das diferenças de carga elétrica entre as moléculas carreadoras e a membrana plasmática – os lipídeos ou polímeros são carregados positivamente

e interagem com a membrana negativa, o que induz a endocitose e a internalização de moléculas exógenas[23]. Ambas as estratégias são eficientes, mas possuem custos distintos: a eletroporação é praticamente sem custo, uma vez que se tenha o equipamento. Já a transfecção química utiliza reagentes geralmente custosos, mas não há a necessidade de ter equipamento especializado. Portanto, a escolha do método depende da disponibilidade de recursos e da frequência com que as transfecções serão feitas no laboratório.

A Tabela 3 ilustra alguns exemplos de linhagens celulares de fácil transfecção que podem ser utilizadas e a eficiência de transfecção esperada com diferentes métodos, de acordo com seus fabricantes. Observe como a eficiência varia consideravelmente entre os métodos; por exemplo, a transfecção das células RAW264 pode variar de <30% a >80%, dependendo do método e das condições utilizadas. Ou seja, para cada novo experimento, as etapas devem ser otimizadas e determinadas empiricamente. Outros fatores, como o tamanho do plasmídeo, também podem influenciar a eficiência (plasmídeos maiores tendem a transfectar menos eficientemente).

Tabela 3: Comparação de eficiência de transfecção entre diferentes métodos de acordo com o fabricante.

	Eletroporação	**Moléculas catiônicas**		
Linhagem celular	**Nucleofector (Lonza)**	**Lipofectamine 3000 (ThermoFisher)**	**FuGENE ou ViaFect (Promega)**	**jetPRIME (Polyplus)**
3T3	51-79%	51-79%	51-79%	51-79%
A549	>80%	51-79%	>80%	NA
C2C12	51-79%	51-79%	>80%	70-90%
Caco-2	>80%	51-79%	NA	<30%
CHO-K1	51-79%	51-79%	NA	51-79%
COS-7	>80%	51-79%	>80%	51-79%
H9c2	>80%	51-79%	>80%	NA
HCT116	>80%	>80%	>80%	51-79%
HEK 293	>80%	>80%	>80%	>80%
HeLa	>80%	>80%	>80%	>80%
HepG2	51-79%	>80%	>80%	51-79%
Hs 578T	51-79%	>80%	NA	NA
Huh-7	51-79%	51-79%	>80%	30-50%
Jurkat	51-79%	<30%	>80%	NA
K-562	>80%	30-50%	NA	NA
L929	>80%	<30%	NA	NA
LNCaP	51-79%	>80%	>80%	NA
MCF7	51-79%	30-50%	>80%	30-50%
Neuro-2a	51-79%	>80%	NA	NA
PANC-1	>80%	51-79%	NA	NA
PC12	>80%	51-79%	>80%	NA
RAW264.7	51-79%	<30%	>80%	30-50%
SH-SY5Y	>80%	<30%	NA	70-80%
SW480	51-79%	51-79%	NA	NA
U2OS	>80%	>80%	>80%	NA
Vero	>80%	30-50%	NA	30-50%

NA: dado indisponível.

Um dos fatores que influenciam diretamente a eficiência da transfecção é o número de células utilizado. A recomendação geral é utilizar células com aproximadamente 70% de confluência; esta medida, porém, pode ser subjetiva e variar entre pesquisadores. A Figura 8 mostra a eficiência de transfecção obtida em nosso laboratório utilizando Lipofectamine® 3000 em diferentes linhagens celulares e com diferente número de células. As células foram transfectadas com um plasmídeo que expressa proteína verde fluorescente (GFP) e a porcentagem de células GFP+ foi avaliada por citometria de fluxo. Para células Hek293, como no exemplo, a eficiência de transfecção aumentou de 40% a 80% com a diminuição do número de células.

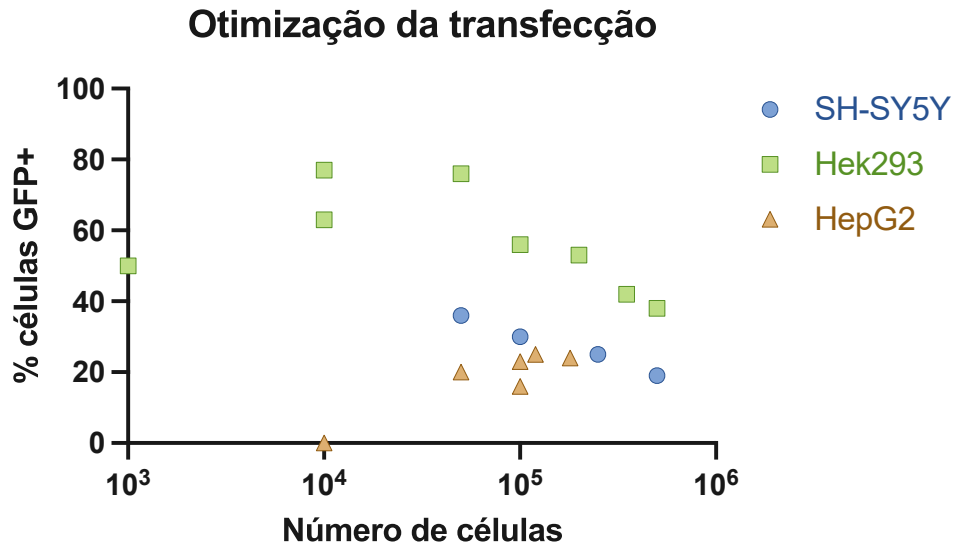

Figura 8: Exemplo do efeito do número de células na eficiência de transfecção de diferentes linhagens celulares usando Lipofectamine 3000.

Nota: As linhagens celulares SH-SY5Y, Hek293 e HepG2 foram plaqueadas em diferentes quantidades e transfectadas em placas de 12 poços, utilizando 1 µg do plasmídeo pIRES-eGFP-Puro, numa proporção de 1,5:1 com Lipofectamine® 3000. Para as linhagens SH-SY5Y e Hek293, é possível observar que a porcentagem de células GFP+ diminui conforme o número de células utilizadas na transfecção foi maior. O número de células não parece interferir dramaticamente na eficiência de transfecção da linhagem HepG2, embora poucas células resultaram em transfecção nula nestas condições. Cada ponto no gráfico representa um experimento distinto.

Os testes de otimização podem ser feitos utilizando poucas células e em placas de cultivo com pouca área. Uma vez estabelecido o melhor protocolo, a transfecção pode ser escalonada para quantidades maiores para realizar o experimento. A Tabela 4 traz o fator de multiplicação para cada frasco de cultivo e, como exemplo de reagente de transfecção, as quantidades de Lipofectamine® 3000. Entretanto, o mesmo fator de multiplicação pode ser utilizado para escalonar experimentos com outros reagentes.

Tabela 4: Escalonando reagentes para transfecção.

Frasco de cultivo	Fator de multiplicação*	Área (cm²)	Volume meio (ml)	Lipofectamine (µl)	DNA (µg)	P3000 (µl)	Opti-MEM (µl)
96 poços	0.16	0.32	0.1	0.12	0.1	0.2	2 x 4.25
48 poços	0.55	1.1	0.3	0.4	0.3	0.6	2 x 12.5
24 poços	1	2	0.5	0.75	0.5	1	2 x 25
12 poços	2	4	1	1.5	1	2	2 x 50
6 poços	4.75	9.5	1.5	3.6	2.4	5	2 x 125
T25	12.5	25	2	9.4	6.25	12.5	2 x 312
T75	37.5	75	10	28.1	18.75	37.5	2 x 986

* O fator de multiplicação apresentado nesta tabela é referente à área do frasco de cultivo e pode ser utilizado para escalonar qualquer experimento envolvendo células em cultivo.

Métodos de isolamento de clones

O isolamento e expansão de clones tem a finalidade de gerar uma população homogênea, geneticamente idêntica. No contexto de um experimento de edição genômica, isso significa que todas as células terão a mesma edição – seja um nocaute ou um *knock-in*.

A maneira mais comum de se fazer isolamento clonal é utilizando citometria de fluxo e sorteamento de célula única (ou *single-cell sorting,* em inglês). Para isso, preparam-se placas de 96 poços com meio de cultivo para receber uma única célula por poço. Após o sorteamento, as células ficam em cultivo por algumas semanas até atingirem confluência suficiente para serem expandidas para placas com área maior. A vantagem desse método é a garantia de obtenção de populações clonais e a baixa manutenção, pois requer somente poucas trocas de meio ao longo de 30 dias. Outro ponto bastante positivo é que, caso utilize-se métodos contendo marcadores de seleção (como GFP, por exemplo), pode-se selecionar somente as células transfectadas para fazer o plaqueamento, o que aumenta a eficiência do processo como um todo. As desvantagens incluem a necessidade de ter o equipamento disponível e a dificuldade de alguns tipos celulares crescerem a partir de células únicas[22,24].

Outra maneira de se isolar clones é por diluição seriada. Para isso, faz-se uma suspensão bem diluída de células e plaqueia-se em placas de 96 poços o equivalente a 0,5 células, ou menos, por poço. Assim, a chance de se obter poços com mais de uma célula diminui consideravelmente. A vantagem desse método é a facilidade de execução, por não requerer equipamento especializado. Entretanto, a principal desvantagem é a possibilidade de, inadvertidamente, plaquear 2 células em um mesmo poço e obter populações mistas[22,24].

Por fim, pode-se plaquear células aderentes em grandes áreas e em baixa densidade. Assim, as células ficarão espalhadas pela área de cultivo e crescerão em algumas colônias isoladas. Quando as colônias apresentarem múltiplas células, transfere-se a colônia para ser expandida em um poço isolado. Esse método funciona melhor para células que não crescem bem quando plaqueadas sozinhas em um único poço, provavelmente por necessitarem de sinalização das células vizinhas; porém, a chance de contaminação de uma colônia clonal com outras células é maior que nos demais métodos[22].

Expansão clonal é necessária?

Nem todos os modelos requerem isolamento de clones e é importante avaliar a sua necessidade antes de iniciar o projeto, uma vez que esta etapa é bastante dispendiosa. Considere o exemplo hipotético: uma doença é causada por deficiência ou ausência completa da enzima B, responsável pela conversão do substrato A no produto C. Com a deficiência de B, há acúmulo de A, que é tóxico. Queremos avaliar a eficácia de duas possíveis terapias:

- Uma molécula que degrada A independente da enzima B;
- Uma molécula que se liga nos aminoácidos alterados da enzima B, modula sua estrutura e aumenta sua atividade.

No primeiro caso, o efeito do tratamento independe da enzima B; ela pode estar com a estrutura alterada ou pode estar completamente ausente e ainda será possível medir o efeito do tratamento, ou seja, a diminuição do componente tóxico A. Neste caso, pode-se gerar uma população mista nocaute para o gene B, contendo células com mutações diversas, desde que a proteína não seja produzida ou não tenha atividade. Já no segundo caso, a enzima B deve estar presente e deve possuir as alterações específicas em sua estrutura para que o efeito da molécula moduladora seja avaliado. Portanto, para este segundo experimento, é importante que todas as células tenham a mesma alteração específica e, assim, o isolamento de clones e a expansão clonal se fazem necessários.

Vantagem ou desvantagem seletiva

Dependendo da alteração a ser induzida nas células, podemos induzir vantagem ou desvantagem seletivas nas células editadas. Caso a edição ocorra em genes relacionados à proliferação celular, por exemplo, as células editadas poderão expandir mais rapidamente do que as não editadas e, assim, dominar o cultivo em algumas passagens e se tornar uma população praticamente homogênea. Por outro lado, caso a edição confira um efeito negativo para a sobrevivência ou proliferação celular, as células editadas diminuirão com o tempo e serão perdidas. Para avaliar se as células possuem vantagem ou desvantagem seletiva, pode-se analisar a proporção de alelos editados ao longo do tempo: 24h, 48h, 72h, 7 e 14 dias, por exemplo. Conforme a cinética esperada de edição (principalmente se utilizado RNP), a proporção de alelos editados pode aumentar em até 72h pós-edição, mantendo-se estável após esse tempo[16].

Validação do modelo

Uma vez obtidas as células editadas, devidamente sequenciadas e com o genótipo confirmado, deve-se validar o modelo. Diversas estratégias podem ser utilizadas, como *western blot* (para demonstrar a ausência ou superexpressão da proteína), expressão gênica (similar ao *western blot*, porém quantificando mRNA), imunofluorescência ou citometria de fluxo (utilizando anticorpos contra a proteína de interesse),

atividade enzimática (caso seja uma enzima a proteína de interesse), ensaios colorimétricos, dentre outros[22].

PARTE III: INICIANDO O PROJETO

Planejamento e desenho experimental

O primeiro passo é a definição do objetivo do projeto e quais perguntas o modelo celular desenvolvido poderá responder. A partir disso, deve-se pensar na estratégia que será usada para edição (qual o gene, se será *knock-in* ou nocaute, se terá mutação específica ou se pode apresentar *indels* variadas) e qual o tipo celular será utilizado. É recomendado utilizar células e genes/proteínas conhecidas pelos pesquisadores para o primeiro projeto do laboratório, o que facilitará no cultivo destas células e nos ensaios funcionais para validar o modelo.

O fluxograma a seguir (Figura 9) resume todas as etapas da criação do modelo e pode servir como norteador para quem estiver iniciando na área.

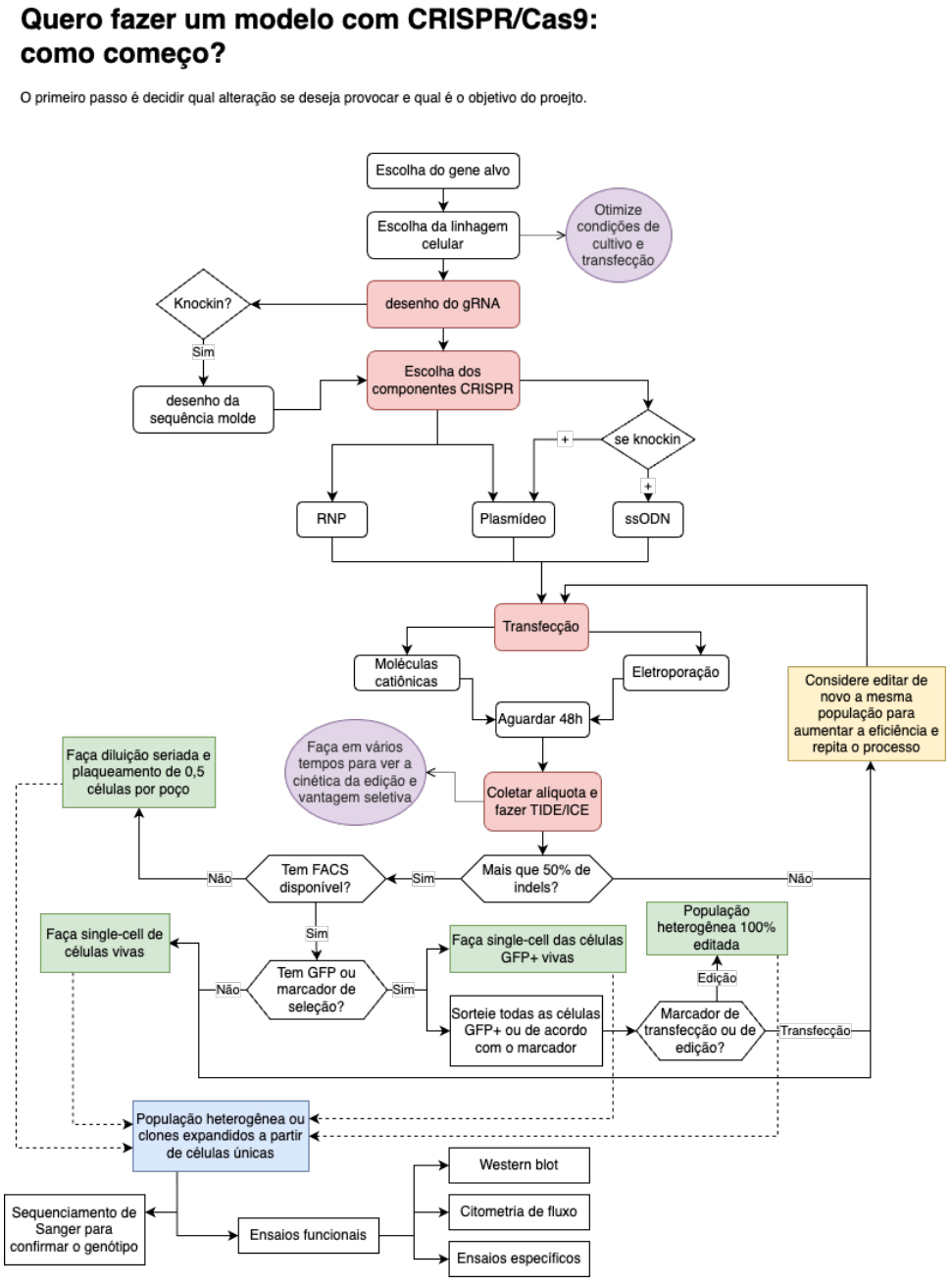

Figura 9: Fluxograma para geração de modelo celular usando CRISPR/Cas9.

A otimização de cada etapa é fundamental

É importante considerarmos a eficiência de cada etapa da criação do modelo. Etapas ineficientes, quando somadas, podem tornar o projeto inviável pelo custo e pelo tempo de execução. Portanto, o planejamento e a otimização de todos os protocolos são necessários para obter a maior eficiência possível.

A Figura 10 ilustra dois experimentos conduzidos com condições otimizadas e condições não-otimizadas. No primeiro experimento, utilizando protocolos padrão e sem otimização, a cada 3.000 células, obtivemos apenas um clone com edição bialélica, o que representou o sequenciamento de 50 clones até um apresentar alterações em ambos os alelos. O mesmo experimento foi repetido utilizando condições otimizadas para o nosso laboratório (gRNA com maior atividade escolhido, entregue à célula como RNP e utilizando protocolo de transfecção otimizado) e um clone com edição bialélica foi obtido a cada 26 células iniciais, sendo que apenas 3 clones precisaram ser sequenciados para identificação do clone de interesse.

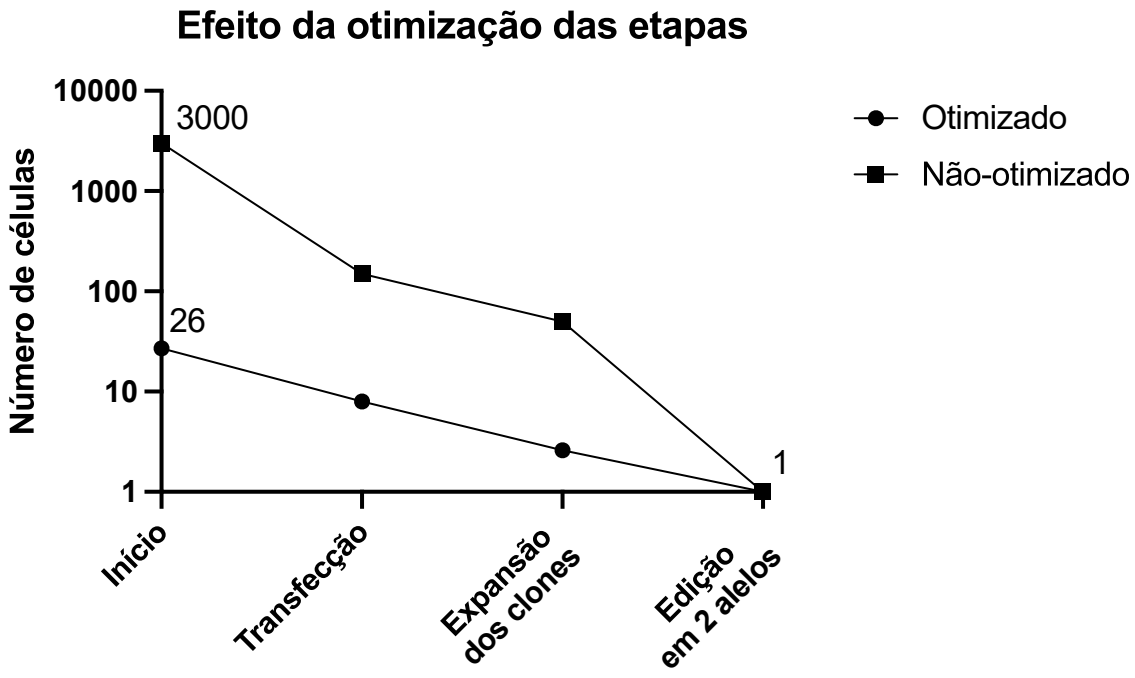

Figura 10: Efeito de cada etapa na eficiência do processo de geração de uma célula nocaute.

Nota: A eficiência de transfecção, de expansão dos clones e de edição bialélica foi de, respectivamente, 5%, 30% e 0,5% para o experimento não-otimizado, e de 30%, 30% e 50% para o otimizado. A otimização pode aumentar a eficiência do processo em até mais de 100 vezes, como no caso exemplificado.

CONCLUSÃO

Baseado na nossa experiência criando diversos modelos nos últimos anos, apresentamos no material suplementar protocolos detalhados de como desenvolver modelos celulares utilizando CRISPR/Cas9. Embora este seja um projeto de meses (estimamos, no mínimo, 12 meses desde a concepção do projeto até a obtenção de células clonais), acreditamos que muitos laboratórios no Brasil têm estrutura e recursos humanos capacitados para desenvolver estes protocolos. Nosso objetivo com esse manuscrito é compartilhar o conhecimento e a nossa experiência no assunto com outros pesquisadores para impulsionar a pesquisa biotecnológica no Brasil.

Leituras recomendadas

Revisão aprofundada sobre CRISPR/Cas9[1];

Capítulo de livro detalhando processos de geração de modelos nocaute com exemplos de modelos já desenvolvidos[22];

Protocolo pioneiro em edição de células eucarióticas, que contém maiores detalhes sobre a etapa de clonagem[25].

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Referências

1. Jiang F, Doudna JA. CRISPR-Cas9 structures and mechanisms. *Annu Rev Biophys*. 2017;46:505-29.
2. Anzalone AV, Koblan LW, Liu DR. Genome editing with CRISPR-Cas nucleases, base editors, transposases and prime editors. *Nat Biotechnol*. 2020;38(7):824-44.
3. Mir A, Edraki A, Lee J, Sontheimer EJ. Type II-C CRISPR-Cas9 biology, mechanism, and application. *ACS Chem Biol*. 2018;13(2):357-65.
4. Sternberg SH, Doudna JA. Expanding the biologist's toolkit with CRISPR-Cas9. *Mol Cell*. 2015;58(4):568-74.
5. Paquet D, Kwart D, Chen A, Sproul A, Jacob S, Teo S, et al. Efficient introduction of specific homozygous and heterozygous mutations using CRISPR/Cas9. *Nature*. 2016;533(7601):125-9.
6. Kwart D, Paquet D, Teo S, Tessier-Lavigne M. Precise and efficient scarless genome editing in stem cells using CORRECT. *Nat Protoc*. 2017;12(2):329-54.
7. McCarty NS, Graham AE, Studená L, Ledesma-Amaro R. Multiplexed CRISPR technologies for gene editing and transcriptional regulation. *Nat Commun*. 2020;11(1):1281.
8. Konstantakos V, Nentidis A, Krithara A, Paliouras G. CRISPR-Cas9 gRNA efficiency prediction: an overview of predictive tools and the role of deep learning. *Nucleic Acids Res*. 2022;50(7):3616-37.
9. Usher I, Ligammari L, Ahrabi S, Hepburn E, Connolly C, Bond GL, et al. Optimizing CRISPR/Cas9 editing of repetitive single nucleotide variants. *Front Genome Ed*. 2022;4:932434.
10. Poletto E, Baldo G, Gomez-Ospina N. Genome editing for mucopolysaccharidoses. *Int J Mol Sci*. 2020;21(2):500.
11. Schubert MS, Thommandru B, Woodley J, Turk R, Yan S, Kurgan G, et al. Optimized design parameters for CRISPR Cas9 and Cas12a homology-directed repair. *Sci Rep*. 2021;11(1):19482.
12. Li K, Wang G, Andersen T, Zhou P, Pu WT. Optimization of genome engineering approaches with the CRISPR/Cas9 system. *PLoS One*. 2014;9(8):e105779.
13. Okamoto S, Amaishi Y, Maki I, Enoki T, Mineno J. Highly efficient genome editing for single-base substitutions using optimized ssODNs with Cas9-RNPs. *Sci Rep*. 2019;9(1):4811.
14. Tham KC, Kanaar R, Lebbink JHG. Mismatch repair and homeologous recombination. *DNA Repair (Amst)*. 2016;38:75-83.
15. Hendel A, Bak RO, Clark JT, Kennedy AB, Ryan DE, Roy S, et al. Chemically modified guide RNAs enhance CRISPR-Cas genome editing in human primary cells. *Nat Biotechnol*. 2015;33(9):985-9.
16. Liang X, Potter J, Kumar S, Zou Y, Quintanilla R, Sridharan M, et al. Rapid and highly efficient mammalian cell engineering via Cas9 protein transfection. *J Biotechnol*. 2015;208:44-53.
17. Xu CF, Chen GJ, Luo YL, Zhang Y, Zhao G, Lu ZD, et al. Rational designs of *in vivo* CRISPR-Cas delivery systems. *Adv Drug Deliv Rev*. 2021;168:3-29.
18. Cromer MK, Vaidyanathan S, Ryan DE, Curry B, Lucas AB, Camarena J, et al. Global transcriptional response to CRISPR/Cas9-AAV6-based genome editing in $CD34^+$ hematopoietic stem and progenitor cells. *Mol Ther*. 2018;26(10):2431-42.
19. Moreb EA, Hutmacher M, Lynch MD. CRISPR-Cas "non-target" sites inhibit on-target cutting rates. *CRISPR J*. 2020;3(6):550-61.
20. Zhang XH, Tee LY, Wang XG, Huang QS, Yang SH. Off-target effects in CRISPR/Cas9-mediated genome engineering. *Mol Ther Nucleic Acids*. 2015;4:e264.
21. Slaymaker IM, Gao L, Zetsche B, Scott DA, Yan WX, Zhang F. Rationally engineered Cas9 nucleases with improved specificity. *Science*. 2016;351(6268):84-8.
22. Poletto E, Baldo G. Creating cell lines for mimicking diseases. *Prog Mol Biol Transl Sci*. 2021;181:59-87.
23. Yip BH. Recent advances in CRISPR/Cas9 delivery strategies. *Biomolecules*. 2020;10(6):839.
24. Giuliano CJ, Lin A, Girish V, Sheltzer JM. Generating single cell-derived knockout clones in mammalian cells with CRISPR/Cas9. *Curr Protoc Mol Biol*. 2019;128(1):e100.
25. Ran FA, Hsu PD, Wright J, Agarwala V, Scott DA, Zhang F. Genome engineering using the CRISPR-Cas9 system. *Nat Protoc*. 2013;8(11):2281-308.

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O panorama da tuberculose em um hospital terciário: perfil clínico-epidemiológico e aspectos da adesão a pontos estratégicos de um protocolo assistencial

The tuberculosis panorama in a tertiary hospital: clinical-epidemiological profile and aspects of adherence to strategic points of a care protocol

Bianca Rocha da Silva[1], Bruno Simas da Rocha[2], Cristófer Farias da Silva[3], Caroline Deutschendorf[1]

RESUMO

Introdução: O estudo objetiva obter o perfil clínico-epidemiológico dos pacientes com tuberculose (TB) em um hospital terciário e verificar a adesão a três pontos estratégicos de um protocolo assistencial - adequado isolamento respiratório na internação de pacientes com TB pulmonar ativa, contrarreferenciamento e orientação farmacêutica com dispensação de tratamento.

Métodos: Estudo transversal realizado em um hospital público e de ensino de Porto Alegre. Foram incluídos os pacientes adultos que tiveram o teste bacilo álcool-ácido resistente (BAAR) e/ou cultura para *Mycobacterium tuberculosis* positivos, entre janeiro de 2018 a dezembro de 2019, com um acompanhamento até abril de 2021. Os fatores relacionados à exposição hospitalar prolongada foram analisados por meio do modelo de regressão multivariada de Poisson.

Resultados: Foram incluídos 122 pacientes. A maioria era do sexo masculino (55,7%), apresentavam a forma pulmonar da doença (88,5%) e foram tratados com o esquema básico exclusivamente durante a internação (82,1%). O contrarreferenciamento foi realizado em 60,8% e a orientação farmacêutica em 54,6% dos pacientes que tiveram alta (n= 97). Na análise dos fatores relacionados com o tempo prolongado fora do isolamento dos pacientes com TB pulmonar ativa (totalizando um n = 144 de internações, considerando as reinternações dos pacientes), foram encontrados como fatores protetores independentes o BAAR positivo, a coinfecção com HIV e o sintoma perda de peso, ao passo que o tempo de internação foi identificado como fator de risco.

Conclusões: Os achados encontrados sugerem que a adesão ao protocolo pode ser intensificada, almejando um cuidado ainda mais integral aos pacientes, minimizando a exposição hospitalar ao bacilo e incrementando o contrarreferenciamento e orientação farmacêutica com dispensação do tratamento na alta.

Palavras-chave: *Tuberculose; alta hospitalar; isolamento de pacientes*

ABSTRACT

Introduction: The study aims to obtain the clinical-epidemiological profile of patients with tuberculosis in a tertiary hospital and to verify adherence to three strategic points of a care protocol - respiratory isolation, counter-referral and pharmacist orientation with treatment dispensing.

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Methods: Historical cohort study carried out in a public teaching hospital in Porto Alegre. Adult patients who had a positive acid-fast bacillus (AFB) stain and/or culture for the *Mycobacterium tuberculosis* Complex between January 2018 and December 2019, with a follow-up until April 2021 were included. Factors related to prolonged hospital exposure (48h or more outside respiratory isolation) were analyzed using Poisson's multivariate regression model.

Results: 122 patients were included. Most were males (55.7%), had the pulmonary form of the disease (88.5%) and were treated with the basic regimen exclusively during hospitalization (82.1%). Counter-referral was performed in 60.8% and pharmacist orientation with treatment dispensing in 54.6% of patients who were discharged (n= 97). In the analysis of factors related to prolonged hospital exposure (n= 144 hospitalizations), positive AFB stain, HIV coinfection and weight loss were found to be independent protective factors, while length of stay was identified as a risk factor.

Conclusions: The findings suggest that adherence to the protocol can be intensified, aiming at an even more comprehensive care for patients, minimizing hospital exposure to the bacillus and increasing counter-referral and pharmacist orientation with treatment dispensing at discharge.

Keywords: *Tuberculosis; hospital discharge; patient isolation*

INTRODUÇÃO

A tuberculose (TB) é uma doença infectocontagiosa responsável por mais de um milhão de mortes anualmente. O pulmão é um dos principais órgãos acometidos e que resulta na forma da doença responsável pela manutenção da cadeia de transmissão[1]. Seu agente etiológico, *Mycobacterium tuberculosis,* tem sua rota de transmissão aérea e alguns ambientes podem favorecer o espalhamento do mesmo como, por exemplo, o hospitalar[2,3].

O tratamento garante, em boa parte dos casos, a cura da TB e a adesão ao mesmo é imprescindível para tal. No Brasil, ele é disponibilizado integralmente e gratuitamente pelo Sistema Único de Saúde (SUS). Apesar disso, ainda é considerado um dos 30 países com alta carga de TB, de acordo com a Organização Mundial de Saúde (OMS)[1]. Em geral, a TB é uma doença de manejo ambulatorial, entretanto, a hospitalização se mantém como uma parcela considerável no cuidado da doença. Em casos mais graves, a internação hospitalar pode permitir o isolamento de pacientes bacilíferos, garantir uma melhor adesão inicial ao tratamento e propiciar o manejo adequado de reações adversas graves[4,5,6]. Em contrapartida, os custos das hospitalizações são altos e muitos hospitais não têm recursos suficientes para o adequado isolamento respiratório dos pacientes com TB pulmonar ativa[7]. Outro aspecto fundamental é a continuidade do tratamento após alta hospitalar. É importante, portanto, o contrarreferenciamento dos pacientes, isto é, a transferência dos cuidados de um serviço de saúde de maior complexidade a um de menor complexidade para o seguimento do tratamento[8].

Uma vez que o cuidado com o paciente acometido com TB persiste desafiador e muitos terão seu diagnóstico feito no serviço de saúde com alta complexidade, a organização desse cuidado deve ser respaldada por protocolo assistencial que garanta as corretas orientações aos profissionais envolvidos no atendimento desses pacientes. Portanto, este estudo se propõe a traçar um perfil clínico-epidemiológico dos pacientes e explorar a adesão a pontos estratégicos de um protocolo assistencial de TB em um hospital público e de ensino.

MÉTODOS

Delineamento

Foi realizado um estudo transversal em um hospital público e de ensino, localizado em Porto Alegre, Rio Grande do Sul, Brasil. O hospital possui 845 leitos, sendo quatro para isolamento de pacientes com TB pulmonar ativa (com pressão negativa). Para orientação do fluxo assistencial, a Instituição possui um Protocolo Assistencial de Tuberculose (PAT), elaborado com base no "Manual de Recomendações para o Controle da Tuberculose no Brasil"[8]. O PAT visa fornecer orientações para a prevenção, o diagnóstico e o tratamento da infecção por *Mycobacterium tuberculosis*.

Foram incluídos no estudo pacientes hospitalizados com 18 anos de idade ou mais, que positivaram a cultura para o complexo *Mycobacterium tuberculosis* (CMT) e/ou a pesquisa para bacilo álcool-ácido resistente (BAAR) em qualquer amostra, entre o período de janeiro de 2018 a dezembro de 2019, com um acompanhamento até abril de 2021.

Coleta de dados e variáveis

A coleta de dados foi realizada a partir dos prontuários eletrônicos dos pacientes com o auxílio de um instrumento de coleta estruturado. Os dados foram tabulados no *Microsoft Excel*®. O "Manual de Recomendações para o Controle da Tuberculose no Brasil"[8] foi utilizado para a determinação das variáveis de interesse. Foram coletadas as seguintes variáveis: idade, sexo, pertencimento a população especial para a TB, amostra, resultados de cultura e/

ou BAAR, sítio da infecção, comorbidades, exames de imagem realizados e os respectivos achados, tempo (em dias) de internação hospitalar, sinais e sintomas identificados na internação, tratamento anti-TB prescrito (esquema, dose e posologia), reações adversas maiores, admissão por qualquer causa no Centro de Tratamento Intensivo (CTI), desfecho hospitalar (alta, óbito por qualquer causa ou transferência para outro serviço de saúde) e, para aqueles que tiveram alta hospitalar, se houve reinternação por qualquer causa enquanto tratando TB.

No que tange a adesão ao protocolo assistencial de TB, foi avaliado o contrarreferenciamento a um serviço de saúde de menor complexidade pelo assistente social ou outro profissional de saúde, e se houve orientação farmacêutica com dispensação de quantitativo do tratamento, visando continuidade do tratamento até a vinculação ao serviço de referência. Referente à adequação ao isolamento respiratório, foram coletados o tempo (em dias) fora do isolamento dos pacientes com TB pulmonar ativa.

Definições

Pacientes com uso de esquema básico foram definidos como aqueles que usaram exclusivamente essa terapia (RHZE/RH)[8], ao passo que pacientes com uso de esquema especial (qualquer abordagem terapêutica que não envolvesse a administração de RHZE/RH, conforme estabelecido pelo Ministério da Saúde)[8], foram definidos como aqueles que usaram esquema especial exclusivamente ou que realizaram troca do básico para o especial.

Para pacientes com reinternação hospitalar, no que diz respeito aos desfechos, coletou-se o desfecho final, bem como se utilizou a média das variáveis contínuas e, se em alguma dessas internações, houve dispensação dos medicamentos e contrarreferenciamento na alta.

Casos de TB pulmonar ativa com tempo prolongado fora de isolamento foram definidos como aqueles pacientes com testes bacteriológicos positivos em uma amostra proveniente do pulmão que permaneceram 48 horas ou mais fora do isolamento respiratório durante a internação hospitalar. Foram contabilizados como “dias fora do isolamento” pacientes que tiveram o isolamento tardio, bem como aqueles que tiveram o isolamento descontinuado precocemente e apresentaram a cultura positiva para CMT posteriormente.

Análises estatísticas

Os dados foram analisados com o software *Statistical Package for the Social Sciences*® versão 25. Variáveis contínuas foram expressas como mediana e intervalo interquartil (IIQ). Para as variáveis categóricas, foram apresentadas as frequências absolutas e relativas. Para a análise utilizando a exposição prolongada como variável dependente, foi realizada análise univariada por regressão de Poisson. Todas as variáveis que tiveram um valor de p menor que 0,05 na análise univariada foram inseridas na análise multivariada, sendo esta realizada pela regressão de Poisson também. Antes dessa última análise, foi realizada uma avaliação da multicolinearidade com ANOVA (modelo de regressão linear) para verificar se as variáveis quantitativas poderiam ser utilizadas no modelo multivariado como explicativas.

Aprovação ética

Este estudo foi aprovado pelo Comitê de Ética em Pesquisa em Seres Humanos da instituição sob o número do Certificado de Apresentação para Apreciação Ética: 43836621.4.0000.5327.

RESULTADOS

Foram identificados 122 casos de TB no hospital com exames bacteriológicos positivos, conforme o fluxo apresentado na Figura 1. Destes, 68 (55,7%) eram do sexo masculino. A mediana da idade foi de 48 anos (IIQ 25-75%= 33,0-63,0) e a maioria dos pacientes eram procedentes de Porto Alegre (n= 78; 69,9%). A Tabela 1 apresenta detalhadamente a caracterização da amostra estudada.

A principal apresentação clínica da TB foi a pulmonar, com 108 casos (88,5%). Destes, 29 casos tinham também algum acometimento em outro sítio. Pacientes com TB exclusivamente extrapulmonar totalizaram 14 (11,5%), com quatro casos de TB pleural, três ganglionares (um desses ganglionar e óssea), dois geniturinários, dois no sistema nervoso central, uma no ouvido, uma óssea e uma abdominal.

Tabela 1: Características da população estudada e adesão aos pontos do protocolo assistencial de tuberculose (n = 122).

Variáveis	N ou mediana	% ou IIQ (25-75%)
Demográficas		
Sexo (masculino)	68	55,7
Idade (anos)	48,5	33,0-63,0

continua....

Tabela 1: Características da população estudada e adesão aos pontos do protocolo assistencial de tuberculose (n = 122).

Origem		
Porto Alegre	78	69,9
Região metropolitana	31	25,4
Outras localizações do Rio Grande do Sul	13	10,6
Pessoa vivendo em situação de rua	10	8,2
Apresentação da TB		
TB pulmonar	108	88,5
TB extrapulmonar	14	11,5
Exames microbiológicos		
BAAR positivo	79	64,7
Cultura positiva para CMT	82	67,2
TB resistente[a]	7/35	20,0
Principais comorbidades[b]		
Tabagismo ativo ou prévio	45	36,9
HIV	40	32,8
Etilismo ativo ou prévio	32	26,2
Uso de drogas ilícitas ativo ou prévio	26	21,3
Diabetes mellitus	17	13,9
Câncer	13	10,6
Transplante (órgão sólido ou medula óssea)	9	7,4
Outra(s)	64	52,5
Aspectos farmacoterapêuticos		
Esquema básico[c]	87/106	82,1
Esquema especial	19/106	15,6
Sem esquema terapêutico prescrito durante internação	16	13,1
Reações adversas maiores	15/106	14,1
Aspectos da internação hospitalar		
Tempo de internação (dias)	13	7,75-21,0
Tratamento intensivo (por qualquer causa)	39	32,0
Alta hospitalar	93	76,2
Óbito por qualquer causa	27	22,1
Transferência a outro serviço de saúde	2	1,6
Reinternação hospitalar por qualquer causa enquanto tratando TB[d]	34/97	35,1
Tempo médio para reinternação (dias)	55	13,75-91,75
Adesão aos pontos estratégicos do protocolo		
Contrarreferenciamento[d]	59	60,8
Orientação farmacêutica e fornecimento de tratamento na alta[d]	53	54,6
Contrarreferenciamento + fornecimento de tratamento[d]	35	36,1
Exposição prolongada de pacientes com TB pulmonar ativa[e]	62/114	54,4

IIQ: intervalo interquartil; TB: tuberculose; BAAR: bacilo álcool-ácido resistente; CMT: Complexo *Mycobacterium tuberculosis*; HIV: vírus da imunodeficiência humana.

[a] 35 pacientes tiveram cultura positiva para o Complexo M. *tuberculosis* e realizaram teste de sensibilidade para os fármacos anti-TB; [b] O mesmo paciente pode ter apresentado mais de uma comorbidade; [c] 106 pacientes tiveram algum tratamento anti-TB prescrito durante a internação; [d] 97 pacientes passaram pelo processo de alta hospitalar em algum momento (93 sobreviventes e 4 que, na reinternação hospitalar, evoluíram a óbito); [e] Houve 114 internações hospitalares de pacientes com TB pulmonar ativa.

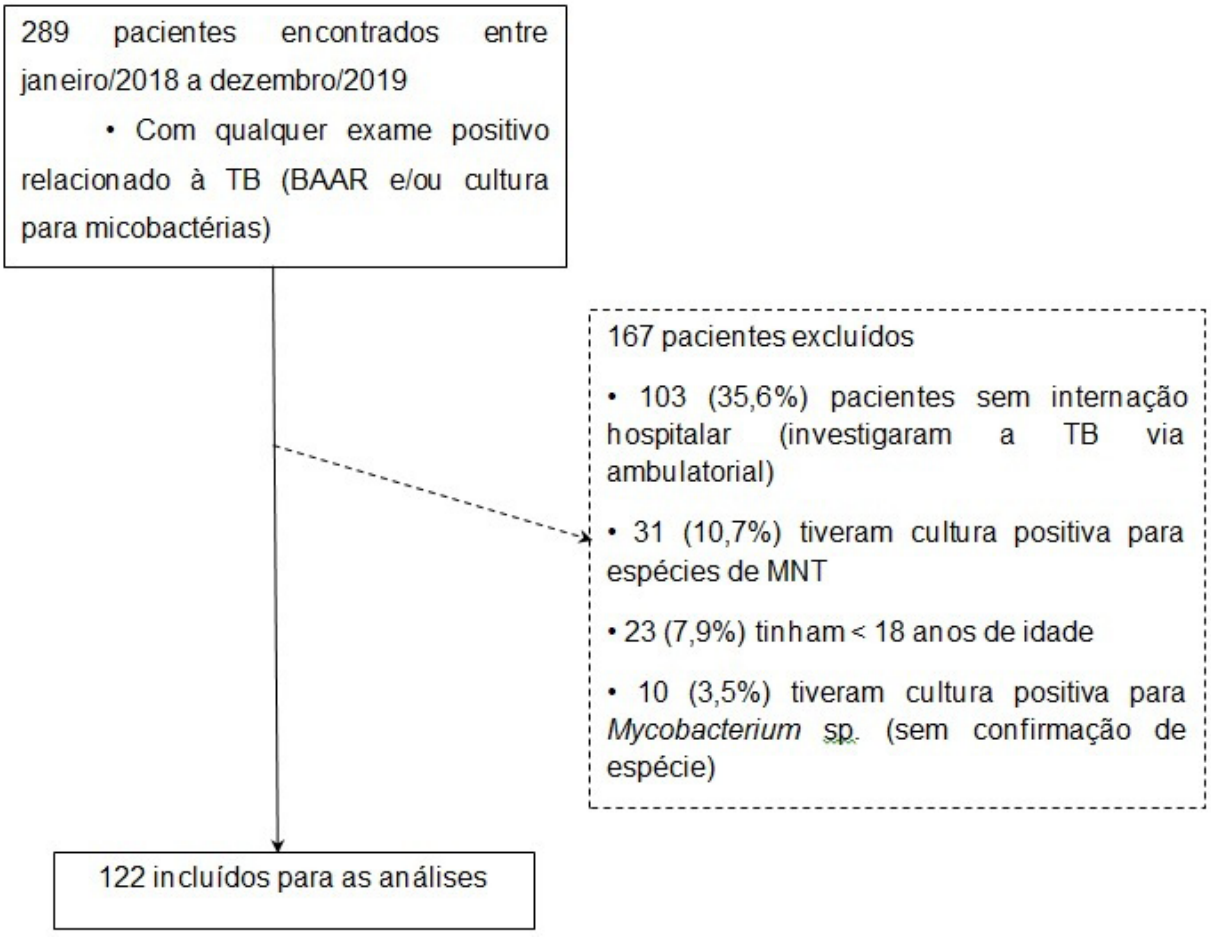

Figura 1: Fluxo de inclusão dos pacientes.

A respeito dos testes microbiológicos, 79 (64,7%) casos tiveram BAAR positivo e 82 (67,2%) tiveram cultura positiva para o CMT. O teste de sensibilidade foi realizado em 35 dessas culturas positivas (conforme os critérios institucionais: pacientes com histórico de abandono de tratamento e/ou HIV positivo). Desses pacientes, 7 (20,0%) apresentaram alguma resistência aos fármacos para tratamento da TB, isto é, apresentaram no teste de sensibilidade ao menos um fármaco como resistente. Houve cinco casos com monorresistência (três à estreptomicina, um à isoniazida e um ao etambutol) e dois casos com multirresistência (um caso resistente à isoniazida e rifampicina, e um caso resistente à isoniazida, rifampicina e pirazinamida).

No que tange às comorbidades, destacam-se como mais frequentes o tabagismo (45 casos; 36,9%) e a coinfecção com o vírus da imunodeficiência humana (HIV) (40 casos; 32,8%). Cabe salientar que 64 casos (52,5%) tinham outras comorbidades em frequências menores (hipertensão arterial sistêmica com 26 casos, coinfecção com o vírus da hepatite C com 13 casos, entre outros agravos como doenças pulmonares, doença renal crônica, obesidade).

Dos 106 pacientes que realizaram terapia anti-TB durante a internação, o uso exclusivo do esquema básico foi feito em 87 (82,1%), e do especial, em 19 pacientes (15,6%) – destes, 15 fizeram troca do esquema básico para o especial durante a internação. A frequência de reações adversas maiores ao tratamento foi de 15 em 106 pacientes (14,1%). A hepatotoxicidade foi a reação adversa mais frequente, acometendo 11 pacientes. Um paciente, além da hepatotoxicidade, apresentou trombocitopenia. Dois pacientes apresentaram nefrotoxicidade, um paciente apresentou exantema moderado e um paciente apresentou duas reações adversas maiores concomitantes – convulsão e pancreatite aguda.

A mediana do tempo de internação foi de 13 dias (IIQ 25-75%= 7,8-21,0). Em relação à internação no CTI por qualquer causa, 39 pacientes (32,0%) necessitaram desse manejo. Quanto aos desfechos hospitalares, o óbito por qualquer causa ocorreu em 27 pacientes (22,1%), ao passo que 93 pacientes

tiveram alta (76,2%) e 2 foram transferidos para outro serviço de saúde (1,6%). A reinternação por qualquer causa enquanto tratando TB ocorreu em 34 dos 97 pacientes que passaram pela alta alguma vez (35,1%). A mediana do tempo para reinternar foi de 55 dias (IIQ 25-75%= 13,8-91,8).

A respeito da adesão aos pontos estratégicos do protocolo assistencial de TB no processo de desospitalização, 59 de 97 (60,8%) pacientes que tiveram alta hospitalar foram contrarreferenciados a um serviço de saúde de menor complexidade – 54 foram realizados pelo assistente social e 5 pelo farmacêutico. Quanto à orientação farmacêutica e dispensação de quantitativo do tratamento, ocorreu em 53 dos 97 pacientes (54,6%).

Em relação ao isolamento dos pacientes com TB pulmonar ativa, houve 114 internações de 107 pacientes com essa apresentação da infecção – isto é, 7 reinternaram ainda apresentando testes microbiológicos positivos. Houve exposição hospitalar prolongada em 62 dessas 114 internações (54,4%). A mediana do tempo fora do isolamento para as internações com exposição prolongada foi de 6 dias (IIQ 25-75%= 3-13,3). Na análise univariada dos fatores determinantes que levaram a uma exposição prolongada de pacientes com TB pulmonar ativa, as variáveis BAAR positivo, sintoma de perda de peso e coinfecção com o HIV foram encontradas como fatores de proteção estatisticamente significativos, ao passo que as variáveis câncer, diabetes mellitus e tempo de internação foram encontradas como fatores de risco estatisticamente significativos. Em uma análise multivariada, apresentada na Tabela 2, BAAR positivo, sintoma de perda de peso e coinfecção com o HIV foram estatisticamente associados a uma redução no risco relativo de exposição prolongada, enquanto o tempo de internação foi significativamente associado como fator de risco para a exposição prolongada.

Tabela 2: Análises dos fatores relacionados com exposição hospitalar prolongada das internações de pacientes com tuberculose pulmonar ativa (n = 114).

Variáveis	Exposição curta (n = 52)	Exposição prolongada (n = 62)	RR (IC 95%)	P valor	RR ajustado (IC 95%)	P valor
Demográficas						
Sexo masculino, n (%)	31 (59,6)	33 (53,2)	0,889 (0,636-1,242)	0,491		
Idade (anos), mediana (IIQ 25-75%)	44 (32,5-55,5)	50 (30,5-64,5)	1,005 (0,996-1,014)	0,277		
Exames microbiológicos, n (%)						
BAAR positivo	48 (92,3)	34 (54,8)	0,474 (0,355-0,632)	0,000	0,562 (0,424-0,744)	0,000
Cultura positiva para CMT	27 (51,9)	42 (67,7)	1,370 (0,939-1,998)	0,102		
TB resistente[a]	3/14 (21,4)	2/14 (14,3)	0,767 (0,245-2,403)	0,649		
Achados de imagem, n (%)[b,c]	**n = 51**	**n = 61**				
Localizados nos lobos superiores	39 (76,5)	42 (68,8)	0,846 (0,596-1,200)	0,349		
Escavação	25 (49,0)	20 (32,8)	0,726 (0,498-1,060)	0,097		
Laudado como sugestivo de TB	32 (62,7)	27 (44,3)	0,713 (0,506-1,005)	0,054		
Principais comorbidades, n (%)[d]						
Tabagismo ativo ou prévio	19 (36,5)	24 (38,7)	1,043 (0,740-1,470)	0,811		
HIV	23 (44,2)	15 (24,2)	0,638 (0,415-0,983)	0,041	0,659 (0,463-0,938)	0,021
Alcoolismo ativo ou prévio	13 (25,0)	19 (30,6)	1,132 (0,795-1,612)	0,490		
Uso de drogas ilícitas ativo ou prévio	15 (28,8)	10 (16,1)	0,685 (0,411-1,141)	0,146		
Diabetes mellitus	3 (5,8)	10 (16,1)	1,494 (1,050-2,126)	0,026	1,287 (0,926-1,787)	0,133

continua....

Tabela 2: Continuação

Câncer	2 (3,8)	8 (12,9)	1,541 (1,074-2,210)	0,019	1,193 (0,764-1,864)	0,438
Transplante (órgão sólido ou medula óssea)	3 (5,8)	4 (6,5)	1,054 (0,542-2,049)	0,876		
Outra(s)	22 (42,3)	36 (58,1)	1,337 (0,946-1,889)	0,100		
Sinais e sintomas identificados, n (%)[e]						
Tosse	34 (65,4)	33 (53,2)	0,798 (0,573-1,112)	0,183		
Febre	23 (44,2)	37 (59,7)	1,332 (0,939-1,890)	0,108		
Dispneia	22 (42,3)	27 (43,5)	1,023 (0,730-1,435)	0,894		
Perda de peso	37 (71,2)	17 (27,4)	0,420 (0,276-0,639)	0,000	0,514 (0,349-0,755)	0,001
Sudorese	13 (25,0)	18 (29,0)	1,095 (0,763-1,572)	0,621		
Perda de apetite	14 (26,9)	16 (25,8)	0,894 (0,661-1,434)	0,894		
Fadiga/fraqueza	12 (23,1)	14 (22,6)	0,987 (0,659-1,478)	0,950		
Dor torácica	13 (25,0)	9 (14,5)	0,710 (0,417-1,209)	0,207		
Hemoptise	5 (9,6)	5 (8,1)	0,912 (0,479-1,737)	0,780		
Outra(s)	21 (40,4)	28 (45,2)	1,092 (0,781-1,528)	0,606		
Aspectos da internação hospitalar						
Equipes infectologia/ pneumologia, n (%)	20 (38,5)	22 (35,5)	0,943 (0,661-1,344)	0,745		
Tempo de internação, mediana (IIQ 25-75%)	11,0 (6-18,75)	14,5 (9-25)	1,010 (1,002-1,018)	0,012	1,013 (1,005-1,020)	0,001

RR: risco relativo; IC: intervalo de confiança, IIQ: intervalo interquartil; BAAR: bacilo álcool-ácido resistente; CMT: Complexo *Mycobacterium tuberculosis*; TB: tuberculose; HIV: vírus da imunodeficiência humana.
[a] 28 internações tiveram cultura positiva para o Complexo M. *tuberculosis* e realizaram teste de sensibilidade para os fármacos anti-TB; [b] 112 internações realizaram exames de imagem; [c] O mesmo paciente pode ter apresentado mais de um achado por exame; [d] O mesmo paciente pode ter apresentado mais de uma comorbidade; [e] O mesmo paciente pode ter apresentado mais de um sintoma.

DISCUSSÃO

O estudo se propôs a traçar um perfil clínico-epidemiológico dos pacientes com TB e a explorar a adesão a três pontos estratégicos de um protocolo assistencial de TB em um hospital terciário: o adequado isolamento respiratório na internação de pacientes com TB pulmonar ativa e, no processo de alta, o contrarreferenciamento a um serviço de saúde de menor complexidade e o fornecimento de terapia anti-TB com orientação farmacêutica.

Observou-se um número elevado de pacientes (n= 122) com casos de TB diagnosticados em um hospital de alta complexidade, em um período curto de inclusão de pacientes no presente estudo. No Brasil, conforme o Programa Nacional de Controle de Tuberculose, recomenda-se o tratamento domiciliar e descentralização do controle da doença para o contexto da atenção básica[8]. A despeito disso, a assistência no ambiente hospitalar não pode ser excluída, até mesmo em países desenvolvidos[9,10]. Um aspecto importante a ser comentado é sobre as implicações de custo ao sistema de saúde público, uma vez que o gasto com a internação hospitalar é alto, podendo representar 65% do total das despesas relacionadas à TB[11].

Quanto à mediana da idade dos pacientes (48 anos; IIQ 25-75% = 33,0-63,0), corrobora com outros estudos nacionais[12,13], reforçando o maior acometimento da população economicamente ativa em países em desenvolvimento, como o Brasil. A respeito do sexo, os homens foram mais

acometidos, com 55,7% dos casos, também sendo congruente com outros estudos[14].

A respeito da continuidade do cuidado, observou-se que aproximadamente 61% dos pacientes foram contrarreferenciados a um serviço de saúde de menor complexidade para seguimento do tratamento. Essa taxa pode indicar que o entendimento dos profissionais envolvidos na assistência da TB hospitalar pode ser intensificado para uma maior obtenção da integralidade da assistência. O adequado contrarreferenciamento é um passo importante para que o paciente siga seu tratamento no domicílio. Essa vinculação é, de acordo com o protocolo assistencial, realizada pelos assistentes sociais que realizam outros papéis importantes, como o encaminhamento de familiares contactantes do paciente bacilífero para rastreio e identificação de potenciais fragilidades para seguimento do tratamento[8].

Quanto ao fornecimento de um quantitativo do tratamento anti-TB e orientação farmacêutica na alta, houve aproximadamente 55% de adesão a este tópico do protocolo. Cabe salientar que esse fornecimento é importante para que o paciente consiga manter o tratamento durante o final de semana, por exemplo. Outra perspectiva, é que esse momento é acompanhado de uma orientação farmacêutica a respeito de aspectos do tratamento, como posologia, possíveis reações adversas, melhor horário para administração, entre outros pontos. Estudos têm demonstrado o papel importante do profissional farmacêutico para adesão ao tratamento da TB[15,16]. O abandono do tratamento da TB afeta o controle da doença e favorece o surgimento da resistência do bacilo aos fármacos[17,18].

O isolamento respiratório de pacientes com suspeita ou confirmação de TB pulmonar ativa é uma medida para o controle da infecção hospitalar[8]. Neste estudo, aproximadamente 54% dos pacientes tiveram tempo prolongado fora do isolamento – 2 dias ou mais fora do isolamento respiratório – quer seja por um isolamento tardio ao internar, quer seja por uma descontinuação precoce. Na análise multivariada, o BAAR positivo foi um fator de proteção independente para exposição hospitalar prolongada, havendo redução de 43,8% no risco relativo. Esse resultado sugere a importância do exame para o manejo dos pacientes, devendo-se atentar para aqueles que o negativam. Há evidências na literatura que sugerem a transmissão da TB em pacientes com BAAR negativo[19,20,21].

Kim e colaboradores examinaram a taxa de atraso ou não isolamento (usando ≥3 dias como parâmetro) de pacientes hospitalizados com TB pulmonar e as causas para tal em um hospital terciário. Eles encontraram que o atraso/não-isolamento ocorreu em 47,7% dos casos. Como fatores de risco independentes associados com a falha no isolamento, encontrou-se a admissão por meio de um ambulatório, a admissão em um departamento que não fosse o de infectologia/pneumologia, achados atípicos no raio X de tórax e o BAAR negativo[22].

Estudo realizado no Japão apresentou fatores associados com o atraso no isolamento de pacientes com TB pulmonar e baciloscopia positiva em um hospital. A mediana do tempo para o isolamento foi de um dia (IIQ 25-75%= 1-4). Na análise multivariada foi encontrado que o tempo para isolamento foi significativamente atrasado para os pacientes de sexo masculino, pacientes sem tosse crônica e em pacientes com lesões não-cavitárias no raio X de tórax[23].

Hsieh e colaboradores avaliaram o risco do atraso para o isolamento em pacientes com TB e esfregaço positivo em departamentos de pneumologia/infectologia, entre outros. Houve atraso para o isolamento em 73,7% dos pacientes. Para pacientes admitidos nos departamentos de infectologia/pneumologia o único fator de risco para o tempo de atraso para isolamento prolongado foi a idade do paciente ser igual ou superior a 70 anos, enquanto nos demais departamentos, os fatores de risco foram achados atípicos no raio X de tórax, sintomas sem dispneia e não ser admitido do departamento de emergência[24].

Em um estudo que objetivou determinar os fatores associados com o isolamento atrasado em pacientes com TB em um hospital de referência, feito por Han e colaboradores, 67% dos pacientes tiveram o isolamento atrasado (após 3 dias da admissão). Na análise multivariada, a idade avançada, admissão a departamentos que não fossem os de pneumologia/infectologia, e a presença de câncer, foram associadas com o isolamento tardio. Eles também encontraram que pacientes com achados radiológicos de TB pulmonar ativa são mais prováveis de serem isolados antes[25].

Percebe-se que os fatores de risco e protetores para o isolamento tardio/exposição prolongada dos pacientes com TB pulmonar bacilífera, no ambiente hospitalar, é variável nos estudos. Há uma falta de padronização na quantidade de dias que são considerados como isolamento tardio/exposição prolongada, sendo alguns autores mais liberais[22,25]. Também, não há consenso quanto às variáveis que devem ser coletadas para análise, havendo uma importante heterogeneidade dos fatores de interesse mostrados nos estudos, o que dificulta a comparação dos resultados entre os mesmos.

Este estudo apresenta limitações. Dada à natureza retrospectiva do delineamento, o uso dos dados reais coletados dos registros em prontuários confere um viés de aferição importante, mesmo tendo sido utilizado um instrumento de coleta padronizado. A inclusão de pacientes exclusivamente com BAAR

positivo também é uma limitação substancial, haja vista que somente a cultura positiva para o Complexo M. *tuberculosis* pode conferir um diagnóstico microbiológico exato de TB. Para uma maior avaliação do impacto dos pontos estratégicos de continuidade do cuidado do protocolo assistencial de TB, em perspectivas futuras, deve-se acompanhar o desfecho dos pacientes pós-alta, verificando se houve continuidade do tratamento, podendo ser usados os sistemas de vigilância epidemiológica nacionais. No que tange a adesão ao protocolo, a realização de uma série histórica pode trazer maiores informações, sob um olhar longitudinal, para a realização de melhorias.

Esse estudo traz informações a respeito da adesão de pontos estratégicos de um protocolo assistencial de TB e informações acerca do perfil clínico-epidemiológico em pacientes de um hospital terciário. Os achados encontrados demonstram a importância do manejo hospitalar em um contexto de pacientes multicomorbidos, que apresentam reações adversas maiores ao tratamento anti-TB, fazem uso de esquemas especiais, passam por tratamento intensivo e, também, apresentam resistência aos fármacos.

Além disso, o estudo reforça a importância da existência de um protocolo assistencial e que há oportunidades de melhorias na adesão nos três pontos estudados: I - isolamento respiratório de pacientes com TB pulmonar ativa na internação, II – contrarreferenciamento; III - orientação farmacêutica na alta hospitalar com possibilidade de fornecimento da terapia. Considerando as características epidemiológicas da TB, especialmente no Brasil, tais dados se revestem de importância para que se possam planejar ações de melhoria que impactem em uma assistência ainda mais integral aos pacientes e que minimizem a exposição hospitalar ao bacilo.

Conflitos de interesse

Os autores declaram não ter nenhum conflito de interesse.

REFERÊNCIAS

1. World Health Organization. Global tuberculosis report [Internet]. Geneva: WHO; 2020 [acesso em 8 jun 2021]. Disponível em: https://www.who.int/publications/i/item/9789240013131.
2. Baussano I, Nunn P, Williams B, Pivetta E, Bugiani M, Scano F. Tuberculosis among health care workers. *Emerg Infect Dis*. 2011;17(3):488-94.
3. Lien LT, Hang NT, Kobayashi N, Yanai H, Toyota E, Sakurada S, et al. Prevalence and risk factors for tuberculosis infection among hospital workers in Hanoi, Viet Nam. *PloS One*. 2009;4(8):e6798.
4. Oscherwitz T, Tulsky JP, Roger S, Sciortino S, Alpers A, Royce S, et al. Detention of persistently nonadherent patients with tuberculosis. *JAMA*. 1997;278(10):843-6.
5. Prasad R, Singh A, Gupta N. Adverse drug reactions in tuberculosis and management. *Indian J Tuberc*. 2019;66(4):520-32.
6. Singleton L, Turner M, Haskal R, Etkind S, Tricarico M, Nardell E. Long-term hospitalization for tuberculosis control experience with a medical-psychosocial inpatient unit. *JAMA*. 1997;278(10):838-42.
7. Zhou Y, Chen C, Jiang H, Pan HQ, Zhu LM, Lu W. High admission rates and heavy inpatient service costs of urban tuberculosis patients in eastern China. *BMC Health Serv Res*. 2019;19:47.
8. Ministério da Saúde (Brasil). Manual de recomendações para o controle da tuberculose no Brasil (2. ed.) [Internet]. Brasília: Ministério da Saúde; 2019 [acesso em 27 mar 2024]. Disponível em: https://www.gov.br/saude/pt-br/assuntos/saude-de-a-a-z/t/tuberculose/publicacoes/manual-de-recomendacoes-para-o-controle-da-tuberculose-no-brasil.pdf/view.
9. Ronald LA, FitzGerald JM, Benedetti A, Boivin JF, Schwartzman K, Bartlett-Esquilant G, et al. Predictors of hospitalization of tuberculosis patients in Montreal, Canada: a retrospective cohort study. *BMC Infect Dis*. 2016;16:679.
10. Taylor Z, Marks SM, Burrows NMR, Weis SE, Stricof RL, Miller B. Causes and costs of hospitalization of tuberculosis patients in the United States. *Int J Tuberc Lung Dis*. 2000;4(10):931-9.
11. Costa JG, Santos AC, Rodrigues LC, Barreto ML, Roberts JA. Tuberculose em Salvador: custos para o sistema de saúde e para as famílias. *Rev Saude Publica*. 2005;39(1):122-8.
12. Bonin CR, Fochat RC, Leite ICG, Pereira TV, Fajardo MO, Pinto CP, et al. Analysis of anti-tuberculosis drug resistance and sociodemographic and clinical aspects of patients admitted in a referral hospital. *Einstein (Sao Paulo)*. 2020;18:eAO4620.
13. Micheletti VCD, Kritski AL, Braga JU. Clinical features and treatment outcomes of patients with drug-resistant and drug-sensitive tuberculosis: a historical cohort study in Porto Alegre, Brazil. *PLoS One*. 2016;11(8):e160109.
14. Belo MTCT, Luiz RR, Hanson C, Selig L, Teixeira EG, Chalfoun T, Trajman A. Tuberculosis and gender in a priority city in the state of Rio de Janeiro, Brazil. *J Bras Pneumol*. 2010;36(5):621-5.
15. Clark PM, Karagoz T, Apikoglu-Rabus S, Izzettin FV. Effect of pharmacist-led patient education on adherence to tuberculosis treatment. *Am J Health Syst Pharm*. 2007;64(5):497-505.
16. Karuniawati H, Putra ON, Wikantyasning ER. Impact of pharmacist counseling and leaflet on the adherence of pulmonary tuberculosis patients in lungs hospital in Indonesia. *Indian J Tuberc*. 2019;66(3):364-9.
17. Fregona G, Cosme LB, Moreira CMM, Bussular JL, Dettoni VV, Dalcolmo MP, et al. Fatores associados à tuberculose resistente no Espírito Santo, Brasil. *Rev Saude Publica*. 2017;51:41.

18. Faustini A, Hall AJ, Perucci CA. Risk factors for multidrug resistant tuberculosis in Europe: a systematic review. *Thorax*. 2006;61(2):158-63.

19. Paranjothy S, Eisenhut M, Lilley M, Bracebridge S, Abubakar I, Mulla R, et al. Extensive transmission of *Mycobacterium tuberculosis* from 9 year old child with pulmonary tuberculosis and negative sputum smear. *BMJ*. 2008;337(7669):a1184.

20. Tostmann A, Kik SV, Kalisvaart NA, Sebek MM, Verver S, Boeree MJ, et al. Tuberculosis transmission by patients with smear-negative pulmonary tuberculosis in a large cohort in the Netherlands. *Clin Infect Dis*. 2008;47(9):1135-42.

21. Lawn SD, Edwards D, Wood R. Tuberculosis transmission from patients with smear-negative pulmonary tuberculosis in sub-Saharan Africa. *Clin Infect Dis*. 2009;48(4):496-7.

22. Kim CJ, Kim Y, Bae JY, Kim A, Kim J, Son HJ, et al. Risk factors of delayed isolation of patients with pulmonary tuberculosis. *Clin Microbiol Infect*. 2020;26(8):1058-62.

23. Nishiguchi S, Tomiyama S, Kitagawa I, Tokuda Y. Delayed isolation of smear-positive pulmonary tuberculosis patients in a Japanese acute care hospital. *BMC Pulm Med*. 2018;18(1):94.

24. Hsieh MJ, Liang HW, Chiang PC, Hsiung TC, Huang CC, Chen NH, et al. Delayed suspicion, treatment and isolation of tuberculosis patients in pulmonology/infectious diseases and non-pulmonology/infectious diseases wards. *J Formos Med Assoc*. 2009;108(3):202-9.

25. Han J, Nam BD, Park SY, Park J, Lee E, Lee EJ, et al. Risk factors for delayed isolation of patients with active pulmonary tuberculosis in an acute-care hospital. *Sci Rep*. 2019;9(1):4849.

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Case Report

A rare case of Gastric Diverticulum in a young patient

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Gastric diverticulum represents a rare disorder of the gastrointestinal system. Although most patients are asymptomatic and the finding of this condition is often incidental, a large diverticulum may be related to upper abdominal pain, dyspepsia, nausea and emesis. In more severe cases, diverticula may cause ulcerations, perforation or even hemorrhage. The diagnosis is usually difficult, the symptoms can be common to a series of other gastrointestinal disorders, turning the anamnesis and physical examination not very specific. Esophagogastroduodenoscopy is recommended for correct visualization of the location and size of the lesion. Surgical resection is considered the main treatment of this condition in symptomatic patients or patients with complications. We describe the case of a young patient with diverticulum in the gastric fundus, and a brief review of the literature.

KEYWORDS: *Gastrointestinal; Gastric diverticulum; Abdominal pain; Dysphagia; Laparoscopic*

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INTRODUCTION

Gastric diverticula were first described by Moebius in 1661 and represent the rarest form of diverticulum in the entire gastrointestinal tract, with an estimated prevalence of 0.04% in studies with diagnosis made by using contrast-enhanced radiographs and 0.02% when autopsies are performed[1,2]. Although it is proportionally similar in men and women, this pathology affects individuals over 40 years of age in approximately 82% of cases and affects young people under 20 years of age in only about 4% of cases[2,3].

CASE REPORT

A 29-years-old female patient with a previous medical history of migraine and previous surgeries for endometriosis and cesarean section, was referred to our service due to recurrent complaints of epigastric pain, nausea and vomiting, without other associated complaints such as fever, weight loss, hematemesis or melena. The patient also denied smoking, alcoholism and drug addiction. Physical and laboratory examination without abnormalities. Abdominal computed tomography (Figure 1) revealed a distended stomach by liquid and gaseous contents with the presence of a gastric diverticulum. The esophagogastroduodenoscopy (EGD) (Figure 2) showed a diverticulum in the gastric fundus with the presence of food remains.

The patient underwent a laparoscopic gastric diverticulectomy (Figure 3). The procedure was performed releasing the greater gastric curvature, the gastric fundus and dissecting the gastric diverticulum with a sealing forceps. The gastric diverticulum was resected completely and with margins, using an endoscopic linear stapler (load 60 mm).

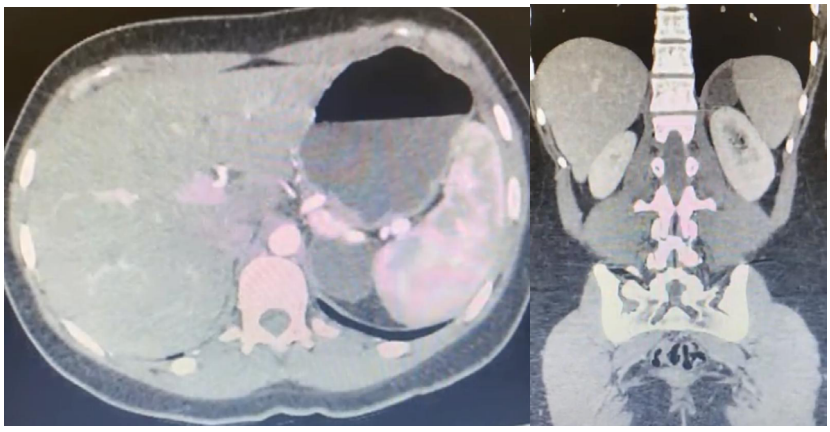

Figure 1: Abdominal computed tomography revealed a distended stomach by liquid and gaseous contents and an hypodense area corresponding to the gastric diverticulum (arrows).

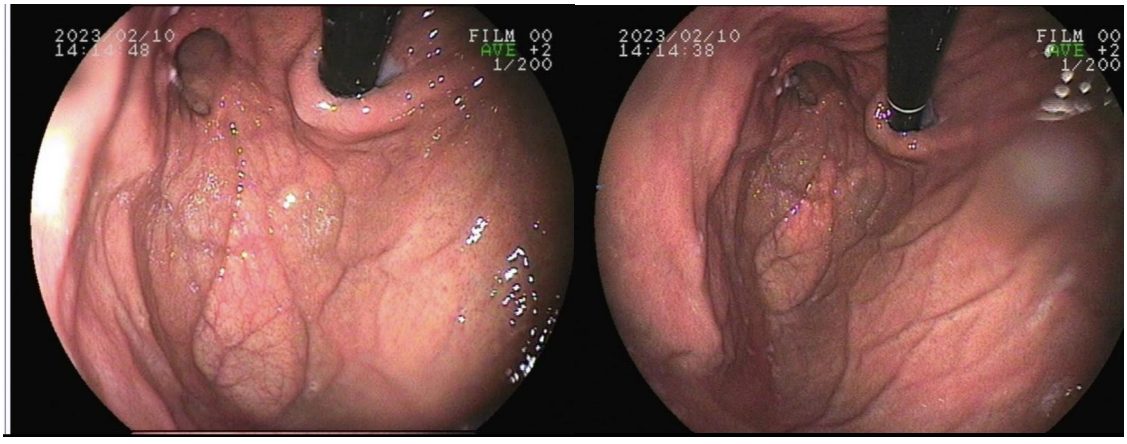

Figure 2: Endoscopic view of the diverticulum in the gastric fundus showing a diverticulum in the gastric fundus with the presence of food remains.

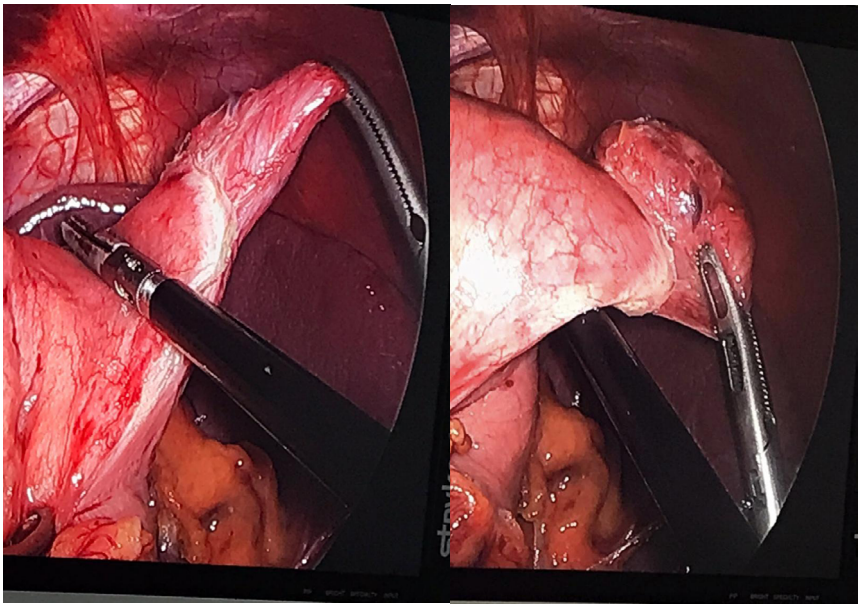

Figure 3: Laparoscopic gastric diverticulectomy.

Finally, the surgical specimen was removed (Figure 4) and sent for anatomopathological examination.

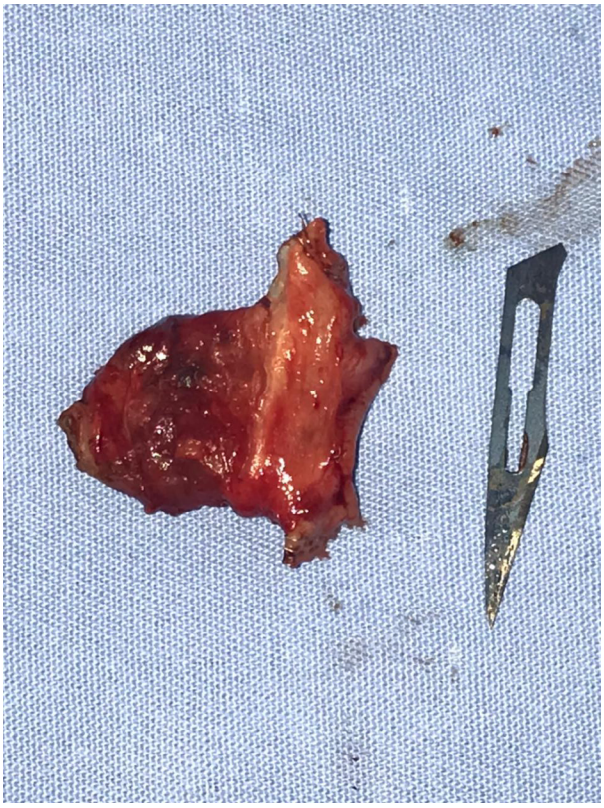

Figure 4: Gastric diverticulum surgical specimen after laparoscopic resection.

DISCUSSION

Gastric diverticulum is unusual and was first described in the literature by Moebius in 1661 and later by Roax in 1774[1]. Its incidence depends on the method used for its observation, and may vary when contrast studies of the upper gastrointestinal tract are performed, with an incidence of 0.04%, and with autopsy studies, with an incidence of 0.02%[4], with only 4% of them occurring in patients younger than 20 years[2]. Even though it is rare, two studies have also shown invasion by gastric adenocarcinoma in the diverticulum[5].

Although most diverticula of the gastrointestinal tract are acquired and originate from the herniation of the mucosa and submucosa through the muscular wall (false diverticula), the gastric diverticulum is usually a true diverticulum, involving all layers of the gastric wall. The gastric diverticula are congenital in 72% of the cases, but acquired form is also possible[4,6].

In addition, gastric diverticula are typically lesions ranging from 1 to 5 cm in diameter, with location and clinical presentation that vary according to their etiology. Thus, it is known that congenital gastric diverticula are commonly found 2 or 3 cm from the gastroesophageal junction along the posterior wall of the stomach, with a symptomatology that is often associated with peptic ulcer disease, gastroesophageal reflux disease, cirrhosis, pancreatitis, hepatitis and cholecystitis[4,7]. Acquired gastric diverticula are located close to the antrum and are generally associated with peptic ulcer disease, pancreatitis, malignancy and gastric obstruction[4,8].

It is important to emphasize that most patients with gastric diverticula remain asymptomatic throughout their lives and this is mainly due to the fact that they usually are true diverticula. However, in the presence of symptoms, it is common to observe epigastric pain, emesis, weight loss, anemia or even more serious complications ranging from obstruction, gastrointestinal bleeding to perforation[2,4,9].

The diagnosis can be confirmed with contrast studies of the upper gastrointestinal tract, computed tomography and esophagogastroduodenoscopy. The vast majority of authors consider the esophagogastroduodenoscopy the gold-standard test for diagnosis, as it can easily identify the location and size of the diverticulum. In addition, the EGD allows biopsy of the lesion[10,11].

The treatment and clinical management of the patient depends on the presence and severity of the symptoms. Thus, gastric diverticula found incidentally or those asymptomatic may not need any specific treatment. Furthermore, the use of therapy with antacids such as proton pump inhibitors for a few weeks may contribute to improve the patient's symptoms[11,12]. In cases of lesions larger than 4 cm, which are most likely to cause complications such as bleeding, perforation or malignancy, the drug therapy is ineffective and surgical intervention is recommended[3,13]. Palmer demonstrated in his study that 6 out of 9 patients with symptoms caused by gastric diverticula who underwent open surgery had excellent results in terms of symptom improvement[14]. Laparoscopic resection of a gastric diverticulum was first described in 1998 by Fine[15]. Apart from that, laparoscopic gastric diverticula resection have been described by several other authors and has been increasingly used because it is considered the safest surgical technique. For this purpose, the most favorable approach and with the most successful results is simple laparoscopic resection of the diverticulum using a stapler[3].

CONCLUSION

Gastric diverticulum is an extremely rare finding among the pathologies that affect the gastrointestinal tract. We describe the case of a young patient, with symptoms of epigastric pain,

nausea, vomiting and with a suspected presence of gastric diverticulum visualized on abdominal computed tomography, which was corroborated by esophagogastroduodenoscopy. Patient underwent a laparoscopic resection of the lesion, with a good postoperative response. This diagnosis must be suspected in patients who typically present with nonspecific gastrointestinal symptoms, with a long history of drug treatments without resolution of the condition. These patients must be followed up with imaging tests, such as esophagogastroduodenoscopy, to allow the visualization of the diverticulum and study its location in order to propose the best therapeutic approach.

REFERENCES

1. Michel ML, Williams WT. Tri-locular gastric diverticulum. *Ann Surg*. 1950;132(2):273-81.
2. Palmer ED. Gastric diverticula. *Int Abstr Surg*. 1951;92(5):417-28.
3. Rashid F, Aber A, Iftikhar SY. A review on gastric diverticulum. *World J Emerg Surg*. 2012;7(1):1.
4. Rodeberg DA, Zaheer S, Moir CR, Ishitani MB. Gastric diverticulum: a series of four pediatric patients. *J Pediatr Gastroenterol Nutr*. 2002;34(5):564-7.
5. Adachi Y, Mori M, Haraguchi Y, Sugimachi K. Gastric diverticulum invaded by gastric adenocarcinoma. *Am J Gastroenterol*. 1987;82(8):807.
6. Gockel I, Thomschke D, Lorenz D. Gastrointestinal: gastric diverticula. *J Gastroenterol Hepatol*. 2004;19(2):227.
7. Cheng EH, Pavelock RR. Multiple gastrointestinal tract diverticula. *Gastrointest Radiol*. 1990;15(1):282-4.
8. Schweiger F, Noonan JS. An unusual case of gastric diverticulosis. *Am J Gastroenterol*. 1991;86(12):1817-9.
9. Palmer ED. Gastric diverticula, with special reference to subjective manifestations. *Gastroenterology*. 1958;35(4):406-8.
10. DuBois B, Powell B, Voeller G. Gastric diverticulum: “a wayside house of ill fame” with a laparoscopic solution. *JSLS*. 2012;16(3):473-7.
11. Marano L, Reda G, Porfidia R, Grassia M, Petrillo M, Esposito G, et al. Large symptomatic gastric diverticula: two case reports and a brief review of literature. *World J Gastroenterol*. 2013;19(36):6114-7.
12. Mohan P, Ananthavadivelu M, Venkataraman J. Gastric diverticulum. *CMAJ*. 2010;182(5):E226.
13. Ramai D, Ofosu A, Reddy M. Gastric diverticula: a review and report of two cases. *Gastroenterol Res*. 2018;11(1):68-70.
14. Palmer ED. Gastric diverticulosis. *Am Fam Physician*. 1973;7(3):114-7.
15. Fine AP. Laparoscopic resection of a large proximal gastric diverticulum. *Gastrointest Endosc*. 1998;48(1):93-5.

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