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Repercussion of thyroid dysfunctions in thyroidology on the reproductive system: *Conditio sine qua non*?

Demet Sengul[1*], Ilker Sengul[2,3], José Maria Soares Junior[4]

Thyroid hormones, i.e., L-thyroxine (3,5,3′,5′-tetraiodothyronine, T4) and L-triiodothyronine (3,5,3′-triiodothyronine, T3), are vital for the normal reproductive function of *Homo sapiens* and animals by affecting directly ovarian, uterine, and placental tissues through specific nuclear receptors, modulating their development and metabolism[1], thus, alteration of the hormonal status of endocrine gland, such as hypothyroidism and hyperthyroidism, may give rise to subfertility or infertility in both humans and animals[2]. Deiodination of T4 via deiodinases (D1, D2, and D3) in the peripheral tissues results in the output of T3 and reverse T3 (rT3). D3 possesses high expression in the uterus, amniotic membrane, and placenta in order not to transfer excessive maternal thyroid hormones toward the growing fetus, while fetal T3 is principally produced by D1 and D2[1,3]. Moreover, the placenta, unlike thyroid-stimulating hormone (TSH), is easily permeable to thyrotropin-releasing hormone (TRH). Maternal TRH, transported to the fetus, has been estimated to play a crucial role in controlling fetal hormonal status prior to the full maturation of the fetal hypothalamic–hypophyseal–thyroid axis, 16th–18th week, 17th day, and 5th–6th week of gestations in human, rat, and sheep, respectively[3,4]. Thyroid hormones are transpired to affect estrous cycle control, maternal ability, ovulation, sexual maturation, maintenance of pregnancy, postnatal maturation, and even lactation via molecular mechanisms. Some authors expressed hypothyroid state as attenuating proliferation of granulosa cells from preantral follicles in rats associated with diminishing the number of nucleolar organizing regions, while some propounded that the granulosa of antral follicles did not exhibit any alteration. The stage of follicular development is substantial for the causal link of hypothyroidism-to-granulosa cell proliferation[1]. *A posteriori*, repercussion of thyroid dysfunctions on the ovaries of *Homo sapiens* and animals have been revealed, *inter alia*, it affects the ovarian activity in rats, *per se*, not only in prepubertal and peripubertal period but also during pregnancy. Furthermore, maternal hormonal dysfunction of this endocrine gland may also influence the offsprings' postnatal ovarian development in rats. Maternal hypothyroidism and hyperthyroidism were asserted to attenuate postnatal follicular progression in the neonatal and prepubertal ovarian activities with a reduction in the numerical data of primordial, primary, secondary, and antral follicles in rats[5]. It has also been reported that thyroid dysfunctions affect luteal vascularization and luteolysis in cyclic and pregnant rats. Although the exact mechanism is unknown, severe hypothyroidism may lead to ovarian cysts in humans and animals through alteration in luteinizing hormone (LH), and preovulatory LH and follicle-stimulating hormone (FSH) levels. Hypothyroidism was demonstrated as attenuating endometrial thickness, the numerical value of endometrial glands, and volume and height of the epithelial layer of the uterus, while hyperthyroidism, in contrast, was reported when the numerical values of secondary and tertiary follicles and corpora lutea were greater, with a reduction of follicular atresia[1,6]. Despite severe hypothyroidism leading to ovarian cysts, the debate is still ongoing in terms of its true causative mechanism[7,8]. Thyroid dysfunctions act on fetal-placental development. However, it depends on the time of onset, associated conception, and severity of the condition. Clinical hypothyroidism and hyperthyroidism necessitate treatment. In addition, it was propounded that the treatment for subclinical hypothyroidism in gestation is useful as it is on the occasion of autoimmune thyroid disease[9,10]. Herewith, hormones

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of this delicate[11-13] endocrine gland are crucial and vital, *sine qua non*, in terms of miscellaneous physiological processes of humans and animals. Furthermore, their alterations in serum concentrations do jeopardize the direct functioning of the living organisms, particularly in the reproductive system. *In fine,* the hormones of *papillon glande thyroïde* are also vital for reproductive purposes. All roads lead to the thyroid.

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AUTHORS' CONTRIBUTION

DS: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration, Resources, Software, Validation, Visualization, Supervision, Writing – original draft, Writing – review & editing. **IS:** Conceptualization, Data Curation, Formal analysis, Investigation, Methodology, Project Administration, Resources, Software, Validation, Visualization, Writing – original draft, Writing – review & editing. **JMSJ:** Conceptualization, Data Curation, Methodology, Project administration, Validation, Visualization, Supervision, Writing – review & editing.

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The use of neutralizing monoclonal antibody in patients with COVID-19: a systematic review and meta-analysis

Suzana Erico Tanni[1*], Diane Rezende Batista[1], Hélio Arthur Bacha[2], Alexandre Naime Barbosa[3], Wanderley Marques Bernardo[4]

INTRODUCTION

Neutralizing monoclonal antibody (mAb) therapies targeting with high specificity to SARS-CoV-2 has been considered as one of the potential therapies for COVID-19 since the beginning of the pandemic. Preclinical studies demonstrated a marked reduction in viral loads in the upper and lower respiratory tract with the use of neutralizing mAbs[1].

The mAbs have the ability to coordinate the immune defense to link to the virus and control the virus load. The mAbs are defined as an antibody derived from a single B-cell clone and recognize a single and unique epitope that can link to their specific epitope on target antigens and can mediate multiple effects such as disruption of the function and eliminate cells or pathogens[2]. These mAbs for COVID-19 are fully human and were discovered from COVID-19 patient' donors, and one of their targets is to block the S protein of the SARS-CoV-2, preventing viral entry into host cells[3].

The U.S. Food and Drug Administration (FDA) issued an emergency use authorization for mAbs to be used as pre-exposure prophylaxis and mild-moderate COVID-19. However, given to the Omicron variant, the FDA did not recommend using casirivimab+imdevimab. In Brazil, mAbs were approved by the Brazilian regulatory agency, i.e., The National Health Surveillance Agency (ANVISA), for use in patients with mild- to-moderate nonhospitalized COVID-19 patients and for the prevention of COVID-19 infection.

This systematic review aimed to identify, describe, evaluate, and synthesize evidence of effectiveness of mAbs in clinical outcomes in COVID-19 patients.

METHODS

This systematic review was conducted following the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) recommendations[4].

Eligibility criteria

The study protocol followed the patients of interest, intervention to be studied, comparison of interventions, and outcome of interest (PICO) methodology. With the use of a mAb as the main study point, the PICO framework was as follows: patients, adult COVID-19 patients; intervention, use of an mAb (casirivimab+imdevimab, bamlanivimab, bamlanivimab+etesevimab, sotrovimab, regdanvimab, and tixagevimab+cilgavimab); comparison between the standard of care (SOC) and placebo; and outcome, symptomatic COVID-19 infection, symptom resolution, adverse event, severe adverse event, hospitalization, and the mortality rate due to any cause in 29 days. The protocol was registered in the PROSPERO International Prospective Register of Systematic Reviews: CRD42022320972.

All phase 3 randomized controlled trials (RCTs) on the topic were included. No restrictions were imposed with regard to the date of publication, language, or availability of the full text of the article.

Information sources and search strategy

Two authors developed a search strategy that was revised and approved by the team, selected information sources, and systematically searched MEDLINE, EMBASE, Central Cochrane, and ClinicalTrials.gov. Specific search strategies were used for each database: ("COVID-19" OR "COVID" OR "coronavirus" OR

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"SARS-CoV-2") AND ("casirivimab and imdevimab" OR "regn-cov2" OR "bamlanivimab etesevimab" OR "Bamlanivimab" OR "Regdanvimab" OR "CT-p59" OR "Sotrovimab" OR "VIR-7831" OR "Tixagevimab and Cilgavimab" OR "Evusheld" OR "AZD7442") AND (therapy/narrow[filter] OR prognosis/narrow [filter] OR comparative study OR comparative studies). Central Cochrane: (COVID-19 OR COVID OR CORONAVIRUS OR SARS-CoV-2) AND (monoclonal antibody).

Study selection

Two researchers independently selected and extracted data from the included studies. First, articles were selected based on their titles and abstracts. Then, the full texts were evaluated to decide whether to include or exclude the studies, and disagreements were resolved by consensus or following a discussion with a third researcher. We performed selection separately by each class of mAbs.

Data collection and investigated outcomes

Data regarding authorship, year of publication, patient description, interventions (anticoagulant and control), absolute numbers of each outcome, and follow-up duration were extracted from the studies by two researchers independently, and the extracted values were compared.

Risk of bias and quality of evidence

The risk of bias for RCTs was assessed using the Cochrane risk of bias (RoB 2) tool[5,6], as were other fundamental elements, and was expressed as very serious, serious, or non-serious. The risk of bias assessment was conducted by two reviewers independently, and in case of disagreement, a third reviewer deliberated the assessment. The quality of the evidence was extrapolated from the risk of bias based on the Grading of Recommendations Assessment, Development and Evaluation (GRADE) terminology as very low, low, or high; for meta-analyses, the GRADEpro Guideline Development Tool ([GDT]; McMaster University, Hamilton, ON, Canada) gives outcomes of very low, low, moderate, or high[7,8].

Synthesis of results and analysis

Categorical outcomes were expressed by group (mAb and control), the number of events, and calculated risk (in %) for each group (by dividing the number of events by the total number of patients in each group). If the risk difference between the groups was significant, a 95% confidence interval (CI) was expressed based on the number needed to treat (NNT) or the number needed to harm. We analyzed separate RCTs that assessed outpatients infected or noninfected COVID-19 patients and hospitalized patients. We analyzed the mAbs separately by molecular type.

We used fixed-effect or random meta-analysis to evaluate the effect of mAbs versus control on the outcomes when these data were available in at least two RCTs. The effects were reported as risk differences (RDs) and corresponding 95%CIs; a 95%CI which encompassed the value 0 in its range indicated that there was no difference in the outcome effect between the mAbs and control arms. RD shows the absolute effect size in the meta-analysis when compared with the relative risk or odds ratio, and this technique can be used when the binary outcome is zero in both study arms. The heterogeneity of the effects among studies was quantified using the I^2 statistic (I^2>50% indicates high heterogeneity). For the meta-analysis, we used Review Manager software, version 5.4 (RevMan 5; Cochrane Collaboration, Oxford, UK)[9].

RESULTS

All characteristics of each study included in this systematic review are presented in Table 1.

Casirivimab+Imdevimab

In total, 103 studies were retrieved from the selected databases. After eliminating duplicates and including studies that met the eligibility criteria, five studies were selected for the assessment of the full texts. Of these, one was excluded (Figure 1). Therefore, four RCTs[10-13] were selected. The characteristics of each study, risk of bias, and quality of evidence are presented in Table 2. We could not perform a meta-analysis regarding the different populations included in each study.

Prevention of COVID-19 infection among previously uninfected household contacts of infected persons

The study randomized 2475 participants (i.e., 1235 in the placebo group and 1240 in the intervention group)[10]. All participants were negative for RT-qPCR with exposure to household index infected persons within 96 h after collection of the index patient's positive COVID-19 test. In the end, 751 participants in the placebo group and 748 participants in the intervention group finalized the study protocol. The intervention group received 1200 mg subcutaneous casirivimab+imdevimab and was stratified by age. The primary end point was the percentage of participants who were symptomatic RT-qPCR during the 28-day efficacy assessment period; the RT-qPCR was collected weekly over 28 days. The RD of the use of casirivimab+imdevimab reduces in 4% the risk of symptomatic and 2% of asymptomatic infection. The adverse event showed an RD reduction in 13% to the casirivimab+imdevimab group compared to placebo (Table 3). The risk of bias was moderate (Table 2) with low quality of evidence.

Table 1. Characteristics of each study included in systematic review.

Study	Design	Population	Intervention (n)	Comparator (n)	Outcome	Time
O'Brien et al.[10]	RCT Double-blind	Adults nonhospitalized without COVID-19 post-exposure	n=748 1200 mg subcutaneous casirivimab+imdevimab	n=751 Placebo	Positive RT-qPCR asymptomatic Positive RT-qPCR symptomatic Adverse event Serious adverse event	28 days
O'Brien et al.[11]	RCT Double-blind	Adults nonhospitalized with early asymptomatic positive COVID-19	n=156 1200 mg subcutaneous casirivimab+imdevimab after 96 h after a collection of the index case's positive	n=158 Placebo	Positive RT-qPCR symptomatic Hospitalization Adverse event Serious adverse event	28 days
Weinreich et al.[12]	RCT Double-blind	Adults non-hospitalized symptomatic positive COVID-19 and risk factors	n=838 Casirivimab+imdevimab (1200 mg) n=1529 Casirivimab+imdevimab (2400 mg)	n=840 Placebo n=1500 Placebo	Hospitalization or death Hospitalization Adverse event Serious adverse event	29 days
Recovery[13]	RCT Open-label	Adults hospitalized in ward with COVID-19 (without of need of respiratory support or cardiac support)	n=2636 Casirivimab 4 g and imdevimab 4 g	n=2636 Standard of care	Death Mechanical ventilation Adverse event	28 days
Cohen et al.[14]	RCT Double-blind	Adults non-hospitalized with negative COVID-19 in skilled nursing and assisted living facility residents and staff	n=484 bamlanivimab 4200mg intravenously	n=482 Placebo	Symptomatic infection Adverse event Death	21 days
Gottlieb et al.[15]	RCT Double-blind	Adults non-hospitalized with COVID-19 with 3 days of onset symptoms	n=104 Bamlanivimab 700 mg n=109 Bamlanivimab 2800 mg n=104 Bamlanivimab 7000 mg n=114 Bamlanivimab 2800 mg +etesevimab 2800 mg intravenously	n=161 Placebo	Symptom improvement Symptom resolution Hospitalization Adverse event Serious adverse event Death	29 days
Dougan et al.[16]	RCT Double-blind	Adults nonhospitalized with COVID-19 and risk factors	n=518 Bamlanivimab 700 mg + etesevimab 1400 mg single dose intravenously	n=517 Placebo	Hospitalization Death Severe adverse event	29 days
Dougan et al.[17]	RCT Double-blind	Adults nonhospitalized with COVID-19 and risk factors	n=520 Bamlanivimab 2800 mg + etesevimab 2800 mg	n=262 Placebo	Hospitalization Death Severe adverse event	29 days
Gupta et al.[18]	RCT Double-blind	Adults non-hospitalized with COVID-19 with 5 days of onset symptoms	n=291 Sotrovimab 500 mg intravenously	n=292 Placebo	Hospitalization Death Severe adverse event	29 days

Development of symptomatic COVID-19 in early asymptomatic COVID-19

The study included 314 asymptomatic with positive RT-qPCR: 156 patients were randomized to receive 1200 mg subcutaneous casirivimab+imdevimab after 96 h after a collection of the index case's positive COVID-19 test sample, and 158 patients were randomized to receive placebo[11]. The primary end point was the proportion of participants who developed signs and symptoms (broad-term) of COVID-19 within 14 days of a positive RT-qPCR at baseline or during the 28-day efficacy assessment period. The RD of the use of casirivimab+imdevimab reduces in 9% the risk of symptomatic infection and 4% of hospitalization. The adverse event showed an RD reduction in 26% to the

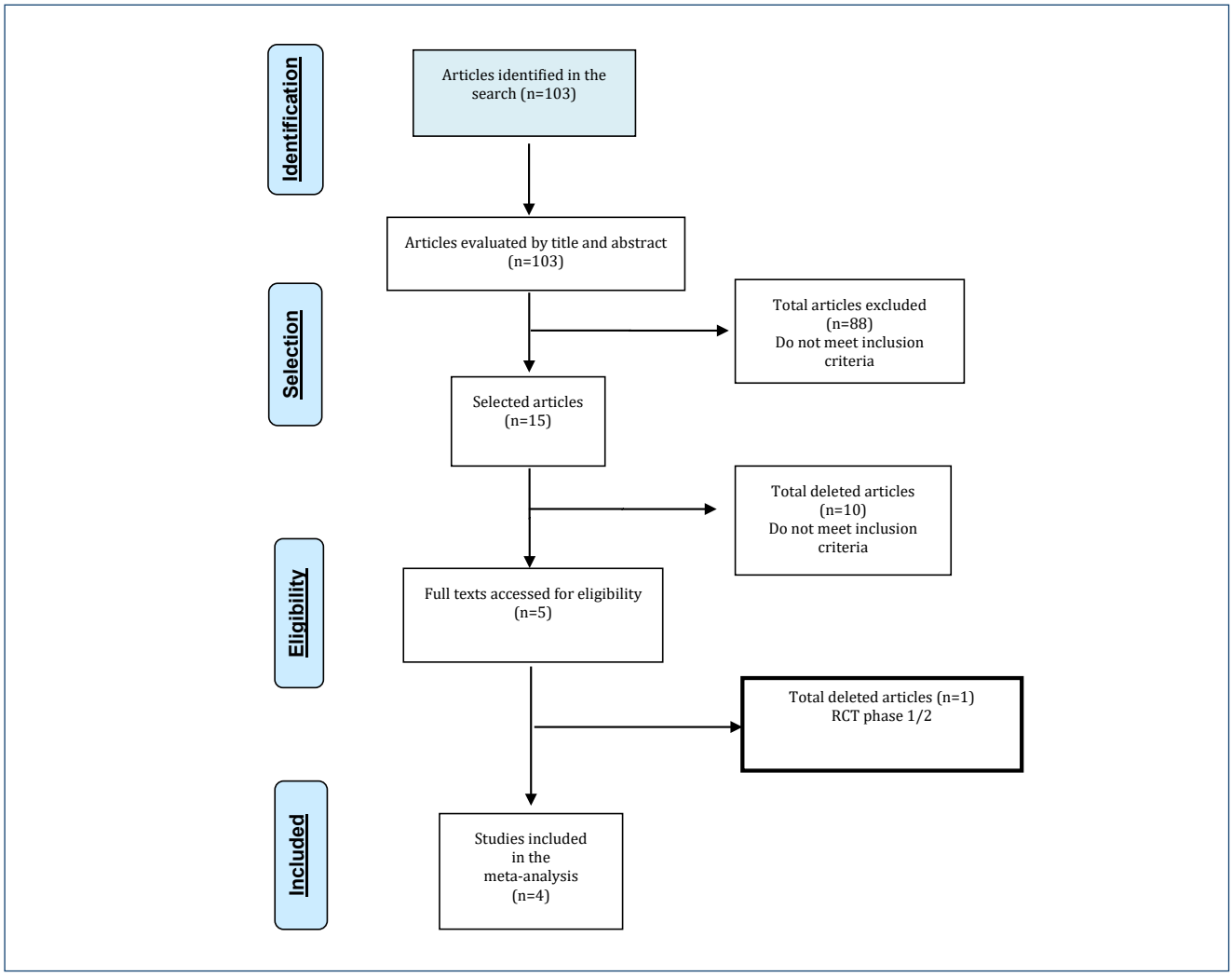

Figure 1. PRISMA flow diagram for casirivimab+imdevimab.

Table 2. Outcomes related to the use of casirivimab+imdevimab compared to placebo in uninfected household contacts of the infected person.

	Placebo n=1235	Casirivimab+imdevimab n=1240	Risk difference (95% confidence interval)
Symptomatic infection	59	11	-0.04 [-0.05; -0.03]
Asymptomatic infection	48	25	-0.02 [-0.03; -0.01]
Adverse event	709	556	-0.13 [-0.16; -0.09]
Serious adverse event	17	14	-0.00 [-0.01; 0.01]

Table 3. Outcomes related to the use of casirivimab+imdevimab compared to placebo in asymptomatic COVID-19-infected person.

	Placebo n=158	Casirivimab+imdevimab n=156	Risk difference (95% confidence interval)
Symptomatic infection in 14 days	44	29	-0.09 [-0.19; 0.00]
Hospitalization	6	0	-0.04 [-0.07; -0.01]
Adverse event	109	67	-0.26 [-0.37; -0.15]
Serious adverse event	4	0	-0.03 [-0.05; 0.00]

casirivimab+imdevimab group compared to placebo (Table 4). The risk of bias was low (Table 2) with low quality of evidence.

Risk of hospitalization or death in outpatients COVID-19-infected persons

This study included outpatients with COVID-19 infection and risk factors. The confirmation of the COVID-19 test needed to be no more than 72 h before randomization with the onset of any COVID-19 symptom no more than 7 days before randomization[12]. The list of risk factors included age >50 years, obesity with body mass index >30 kg/m^2, immunocompromised, diabetes, and liver, kidney, cardiovascular, or lung dysfunction. The casirivimab+imdevimab was administered intravenously, and the primary end point was the percentage of patients with at least one COVID-19-related hospitalization or death from any cause through day 29. The original phase 3 portion of the trial included 3088 patients, with or without risk factors for severe COVID-19, who were randomly assigned to receive a single intravenous dose of casirivimab+imdevimab (8000 or 2400 mg) or placebo. In the amended phase 3 portion of the trial, an additional 2519 patients with at least one risk factor for severe COVID-19 were randomly assigned to receive a single dose of casirivimab+imdevimab (2400 or 1200 mg). The total placebo group was 1500 patients, casirivimab+imdevimab 1200 mg was 838 patients, and casirivimab+imdevimab 2400 mg was 1529 patients randomized. Both doses of 1200 and 2400 mg of casirivimab+imdevimab presented a reduction of hospitalization and death (Table 5) with low risk of bias (Table 2) and low quality of evidence.

Risk of death and mechanical ventilation in hospitalized COVID-19 patients

This study RECOVERY is a randomized, controlled, open-label platform trial comparing several possible treatments with usual care in patients admitted to hospital with COVID-19[13]. Patients admitted to the hospital were eligible for the study if they had clinically suspected or laboratory-confirmed COVID-19 infection. They were assigned (1:1:1) to either the usual standard of care, the usual standard of care plus casirivimab+imdevimab, or the usual standard of care plus convalescent plasma (until January 15, 2021). The intervention group received intravenously casirivimab 4 g and imdevimab 4 g. The primary outcome was 28-day all-cause mortality. Secondary outcomes were time to discharge from hospital and, in patients not on invasive mechanical ventilation at randomization, the composite outcome of invasive mechanical ventilation (including extracorporeal membrane oxygenation) or death. Concomitant medication was predominantly of a systemic corticosteroid, 94% of the total included population, 24% used remdesivir, 14% tocilizumab, and, 9% in both groups used baricitinib. The

Table 4. Outcomes related to the use of casirivimab+imdevimab compared to placebo in outpatient symptomatic COVID-19-infected person.

	Placebo Compare 1200 mg n=840	Casirivimab+imdevimab 1200 mg n=838	Risk difference (95% confidence interval)	Placebo Compare 2400 mg n=1500	Casirivimab+imdevimab 2400 mg n=1529	Risk difference (95% confidence interval)
Hospitalization or death	24	7	-0.02 [-0.03; -0.01]	62	18	-0.03 [-0.04; -0.02]
Hospitalization	23	6	-0.02 [-0.03; -0.01]	59	17	-0.03 [-0.04; -0.02]
Death	1	1	0.00 [-0.00; 0.00]	3	1	-0.00 [-0.00; 0.00]
Adverse event	–	59	–	189	142	-0.03 [-0.06; -0.01]
Serious adverse event	–	9	–	74	24	-0.03 [-0.05; -0.02]

Table 5. Outcomes related to the use of casirivimab+imdevimab compared to placebo in hospitalized COVID-19-infected person.

	Placebo Total n=4946 COVID-19 positive n=2636	Casirivimab+imdevimab Total n=4839 COVID-19 positive n=2636	Risk difference (95% confidence interval)
Death	384	410	0.01 [-0.01; 0.03]
Mechanical ventilation or death	416	459	0.02 [-0.00; 0.04]
Adverse event in positive and negative COVID-19	1715	1792	0.02 [0.00; 0.04]

use of casirivimab+imdevimab increased the risk of mechanical ventilation or death by 2% (Table 6). The risk of bias was high (Table 2) with very low quality of evidence.

Bamlanivimab with or without Etesevimab

In total, 210 studies were retrieved from the selected databases. After eliminating duplicates and including studies that met the eligibility criteria, eight studies were selected for the assessment of the full texts. Of these, three were excluded by the same population, one used combined therapy, and one was phase 2 RCT (Figure 2). Therefore, four RCTs (14-17) were selected. The risk of bias is presented in Table 7. We stratified studies according to the use of the different interventions, such as bamlanivimab isolate or combined to etesevimab.

Bamlanivimab

For the bamlanivimab used alone, we identified two studies[14-15]. We could not perform a meta-analysis regarding the different populations included in each study.

Prevention of COVID-19 infection among previously uninfected contacts of infected persons

The study randomized 1175 participants to evaluate the efficacy of bamlanivimab (4200 mg intravenously – single dose) in skilled nursing and assisted living facility residents and staff (i.e., 587 in the placebo group and 588 in the intervention group) after one positive COVID-19 case at the facility (Cohen JAMA). Within 7 days of a reported confirmed SARS-CoV-2 case at a facility, residents and staff of the facility were screened for enrollment, all participants collected RT-PCR and were randomized to receive intervention or placebo before knowing the results of RT-PCR. The primary end point was the cumulative incidence within 8 weeks of randomization of COVID-19 and the presence of mild or worse disease severity within 21 days of detection. The intervention group and control group with previous negative RT-PCR test was 484 and 482 patients, respectively.

The RD of the use of bamlanivimab reduces in 7% the risk of symptomatic infection. The adverse event or death did not show an RD reduction in the bamlanivimab group compared to placebo (Table 8). The risk of bias was moderate (Table 7) with low quality of evidence.

Risk of hospitalization in outpatients COVID-19-infected persons

This phase 2/3, randomized, double-blind, placebo-controlled, single-infusion study included patients with recently diagnosed mild or moderate COVID-19 in the outpatient setting[15]. All patients were aged 18 years or older, who were tested positive for COVID-19 infection 3 days before randomization with one or more mild-to-moderate symptoms. The main outcome was the SARS-CoV-2 log viral load from baseline to 11 days. The secondary outcomes were time to symptom improvement, time to symptom resolution, the proportion of patients with a COVID-19-related hospitalization, emergency department visit, or death at day 29. Three different doses of bamlanivimab intravenously single dose were used: 700 mg (104 randomized patients), 2800 mg (109 randomized patients), and 7000 mg (104 randomized patients). The placebo group was compound with 161 randomized patients. The proportion of symptom improvement, resolution, hospitalization, and adverse events are presented in Table 9. The use of bamlanivimab reduced by 4% in the hospitalization rate (-0.08 to -0.00). The risk of bias was low (Table 7) with low quality of evidence.

Bamlanivimab+Etesevimab

Risk of hospitalization or death in outpatients COVID-19-infected persons

Three studies assessed this population. One study was phase 2/3, randomized, double-blind, placebo-controlled, single-infusion study that included patients with recently diagnosed mild or moderate COVID-19 in the outpatient setting[15]. All patients aged 18 years or older and were tested positive for

Table 6. Risk of bias of the casirivimab+imdevimab studies included in the systematic review.

RoB 2 Risk of bias from RCT										
Study	Randomization	Allocation	Double blind	Observer	Looses	Charac Prog	Outcome	ITT	Sample size calculation	Early stop trial
O'Brien et al.[10]										
O'Brien et al.[11]										
Weinreichet al.[12]										
Recovery[13]										

RoB 2: Cochrane risk of bias; RCT: Randomized control trial; Charact Prog: Characteristic Prognosis; ITT: Intention to treat.

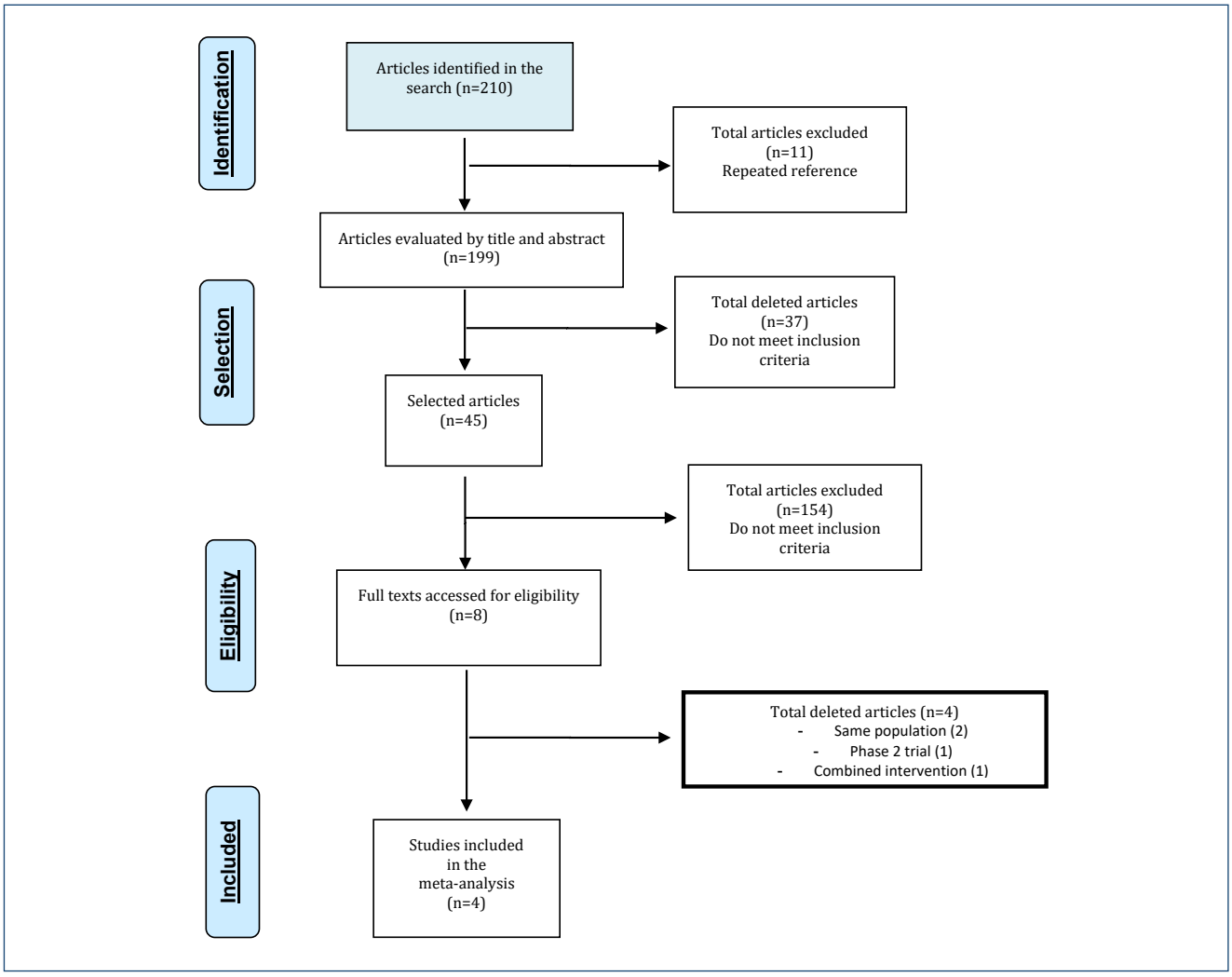

Figure 2. PRISMA flow diagram for bamlanivimab+etesevimab.

Table 7. Outcomes related to the use of prophylaxis bamlanivimab compared to placebo.

	Placebo N=482	Bamlanivimab N=484	Risk difference (95% confidence interval)
Symptomatic infection	73	41	-0.07 [-0.11; -0.03]
Adverse event	86	97	0.02 [-0.03; 0.07]
Death	6	5	-0.00 [-0.02; 0.01]

Table 8. Outcomes related to the use of bamlanivimab in different doses compared to placebo in outpatient symptomatic COVID-19-infected person.

	Placebo N=161	Bamlanivimab 700 mg+2800 mg+7000 mg N=317	Risk difference (95% confidence interval)
Symptom improvement at day 22	96	210	0.07 [-0.03; 0.16]
Symptom resolution at day 22	88	193	0.06 [-0.03; 0.16]
Hospitalization in 29 days	9	5	-0.04 [-0.08; -0.00]
Adverse event	42	75	-0.02 [-0.11; 0.06]
Serious adverse event	1	0	-0.01 [-0.02; 0.01]
Death	0	0	0.00 [-0.01; 0.01]

COVID-19 infection 3 days before randomization with one or more mild-to-moderate symptoms. The main outcome was the SARS-CoV-2 log viral load from baseline to 11 days. The secondary outcomes were time to symptom improvement, time to symptom resolution, the proportion of patients with a COVID-19-related hospitalization, emergency department visit, or death at day 29. The intervention group used bamlanivimab 2800 mg+etesevimab 2800 mg intravenously single dose and compared to placebo. No deaths occurred during the RCT period. We observed the RD of the use of bamlanivimab+etesevimab compared to placebo in outpatient symptomatic COVID-19-infected persons in the recovery of symptom, adverse event, and hospitalization risk.

Other two RCTs[16-17] were performed in outpatients who were 12–17 years of age and who had at least one of the following risk factors at the time of screening: a BMI in at least the 85th percentile for age and sex; sickle cell disease; congenital or acquired heart disease; neurodevelopmental disorders such as cerebral palsy; dependence on a medical-related mechanical device or procedure such as tracheostomy, gastrostomy, or positive-pressure ventilation (not related to COVID-19); asthma, a reactive airway, or another chronic respiratory disease; type 1 or type 2 diabetes mellitus; and an immunocompromised condition or receipt of immunosuppressive treatment. Outpatients who were at least 18 years of age and who presented with at least one of the following risk factors were also included: age of at least 65 years, a BMI of at least 35 kg/m^2, chronic kidney disease, type 1 or type 2 diabetes mellitus, immunosuppressive disease or receipt of immunosuppressive treatment, and an age of at least 55 years with cardiovascular disease, hypertension, or chronic obstructive pulmonary disease or another chronic respiratory disease. All patients had mild or moderate COVID-19 infection confirmed by RT-PCR within 3 days after they had tested positive. The primary outcome was hospitalization (acute care for ≥24 h) or death from any cause by day 29. The difference between studies was the doses of bamlanivimab+etesevimab. One RCT used bamlanivimab 700 mg + etesevimab 1400 mg single dose intravenously, and other RCT used bamlanivimab 2800 mg + etesevimab 2800 mg.

The risk of hospitalization or death was statistically different between groups, with an RD of 5% (95%CI -0.09 – -0.01, p<0.0001, I^2=0%) and NNT=20 in bamlanivimab+etesevimab group (Figure 3A). The risk of bias was low with moderate quality of evidence. Death showed an RD of 2% (95%CI -0.02 – -0.01, p<0.0006, I^2=56%) and NNT=50 in bamlanivimab+etesevimab group (Figure 3B). The risk of bias was low with low quality of evidence. Adverse events or severe adverse events did not demonstrate a difference between groups (Figure 3C and D). The risk of bias was low with low quality of evidence (Table 7).

Sotrovimab

In total, 60 studies were retrieved from the selected databases. After eliminating duplicates and including studies that met the eligibility criteria, two studies were selected for the assessment of the full texts. Of these, one was excluded by using combined therapy intervention (Figure 4).

The RCT included[18] was a double-blind, randomized, for outpatients with symptomatic COVID-19 with ≤5 days after the onset of symptoms, and at least one risk factor for disease progression to receive a single infusion of sotrovimab at a dose of 500 mg or placebo. Patient's high risk for progression of COVID-19 was considered when they presented: older age (≥55 years) or because they had at least one of the following risk factors: diabetes for which medication was warranted, obesity (>30 kg/m^2), chronic kidney disease, congestive heart failure, chronic obstructive pulmonary disease, and moderate-to-severe asthma. The primary efficacy outcome was hospitalization (for >24 h) for any cause or death within 29 days after randomization. The RCT had an early stop trial, because of efficacy, with 583 patients who completed the trial. The safety was performed with 868 patients who were under the protocol at the stop trial. The hospitalization was RD of 6% (95%CI -0.09 – -0.03) and NNT=16.7 of sotrovimab use compared to the placebo group, and severe adverse event RD of 4% (95%CI -0.07 – -0.02) and NNT=25 (Table 10), with the risk of bias moderate with low quality of evidence (Table 11).

Regdanvimab

In total, 30 studies were retrieved from the selected databases. After eliminating duplicates and including studies that met the eligibility criteria, no one study was selected for the assessment of the full texts (Figure 5). We have not identified RCT available at the moment with the eligibility criteria proposed in this systematic review that would support the assessment of the efficacy of regdanvimab in COVID-19 patients.

Tixagevimab+Cilgavimab

In total, 25 studies were retrieved from the selected databases. After eliminating duplicates and including studies that met the eligibility criteria, no one study was selected for the assessment of the full texts (Figure 6). We have not identified RCT available at the moment with the eligibility criteria proposed in this systematic review that would support the assessment of the efficacy of tixagevimab+cilgavimab in COVID-19 patients.

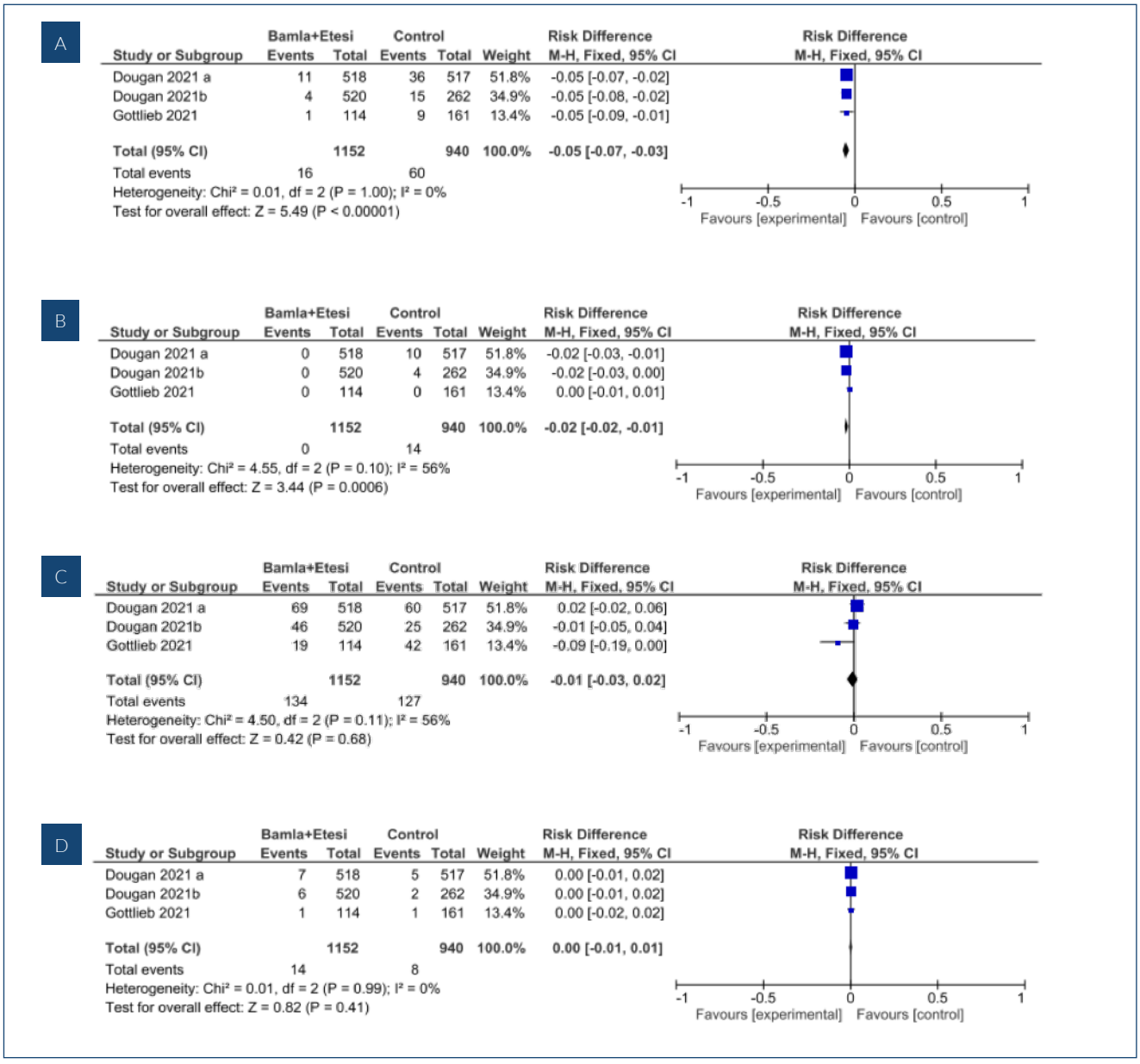

Figure 3. Forest plot of comparison: bamlanivimab+etesevimab versus placebo/SOC (control), with outcome (**A**): hospitalization or mortality in 29 days, (**B**) mortality in 29 days, (**C**) adverse events, and (**D**) severe adverse events.

Recommendations

In patients nonhospitalized without COVID-19:

- The use of casirivimab+imdevimab reduces in 4% the risk of symptomatic COVID-19 infection.
- The use of bamlanivimab reduces in 7% the risk of symptomatic COVID-19 infection.

In patients nonhospitalized with asymptomatic COVID-19:

- The use of casirivimab+imdevimab reduces in 9% the risk of symptomatic infection, 4% of hospitalization, and 26% of the adverse event.

In patients nonhospitalized with symptomatic COVID-19:

- Both doses of 1200 and 2400 mg of casirivimab+imdevimab presented a reduction in hospitalization and death.

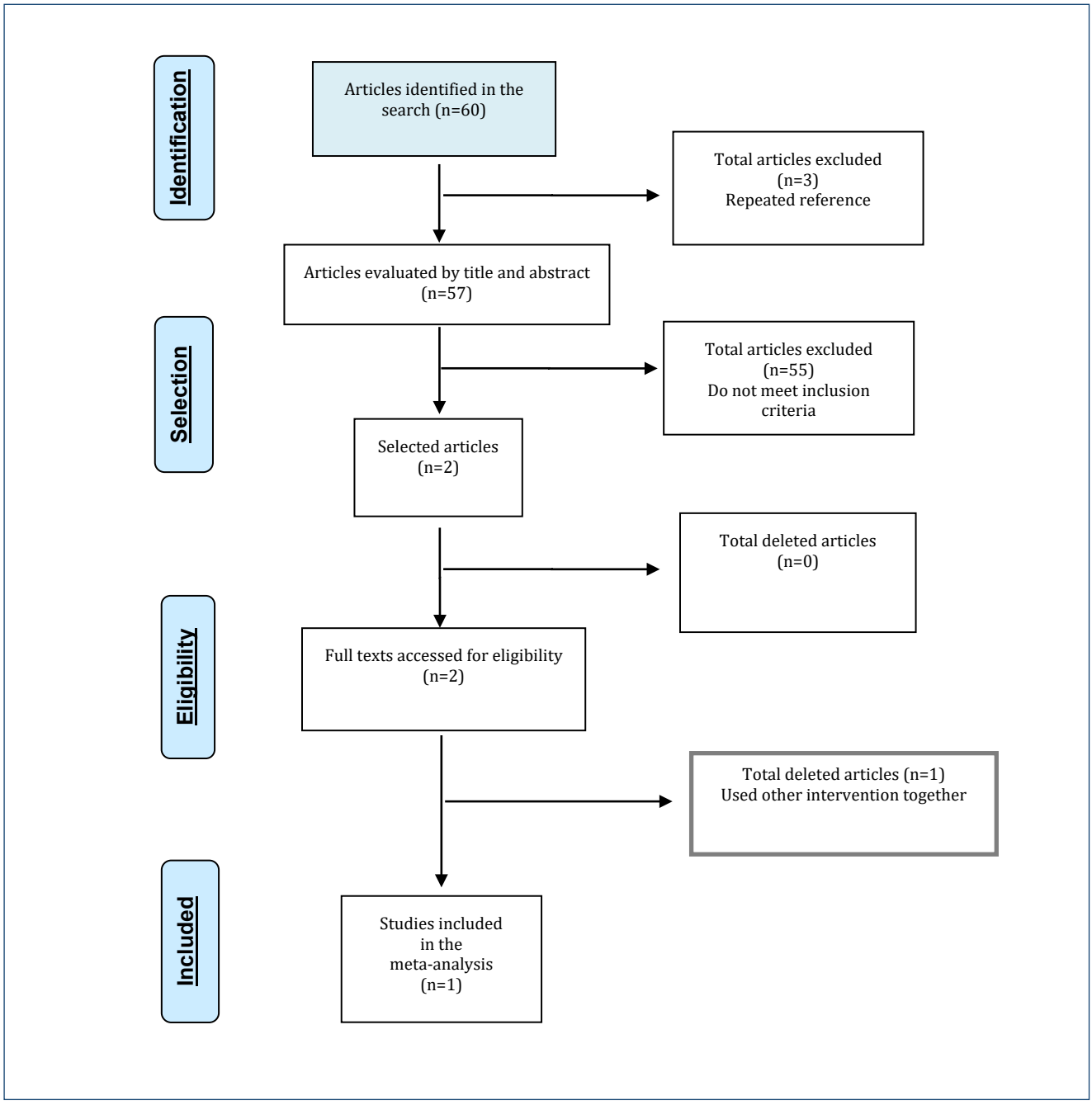

Figure 4. PRISMA flow diagram for sotrovimab.

Table 9. Risk of bias of the bamlanivimab+etesevimab studies included in the systematic review.

RoB 2 Risk of bias from RCT										
Study	Randomization	Allocation	Double blind	Observer	Looses	Charac Prog	Outcome	ITT	Sample size calculation	Early stop trial
Cohen et al.[14]										
Gottlieb et al.[15]										
Dougan et al.[16]										
Dougan et al.[17]										

RoB 2: Cochrane risk of bias; RCT: Randomized control trial; Charact Prog: Characteristic Prognosis; ITT: Intention to treat.

Table 10. Outcomes related to the use of prophylaxis sotrovimab compared to placebo.

	Placebo N=292	Sotrovimab N=291	Risk difference (95% confidence interval)
Hospitalization	21	3	-0.06 [-0.09; -0.03]
Death	1	0	-0.00 [-0.01; 0.01]
Adverse event	85/438	73/430	-0.02 [-0.08; 0.03]
Severe adverse event	26/438	7/430	-0.04 [-0.07; -0.02]

Table 11. Risk of bias of the sotrovimab studies included in the systematic review.

RoB 2 Risk of bias from RCT										
Study	Randomization	Allocation	Double blind	Observer	Looses	Charac Prog	Outcome	ITT	Sample size calculation	Early stop trial
Gupta et al.[18]										

RoB 2: Cochrane risk of bias; RCT: Randomized control trial; Charact Prog: Characteristic Prognosis; ITT: Intention to treat.

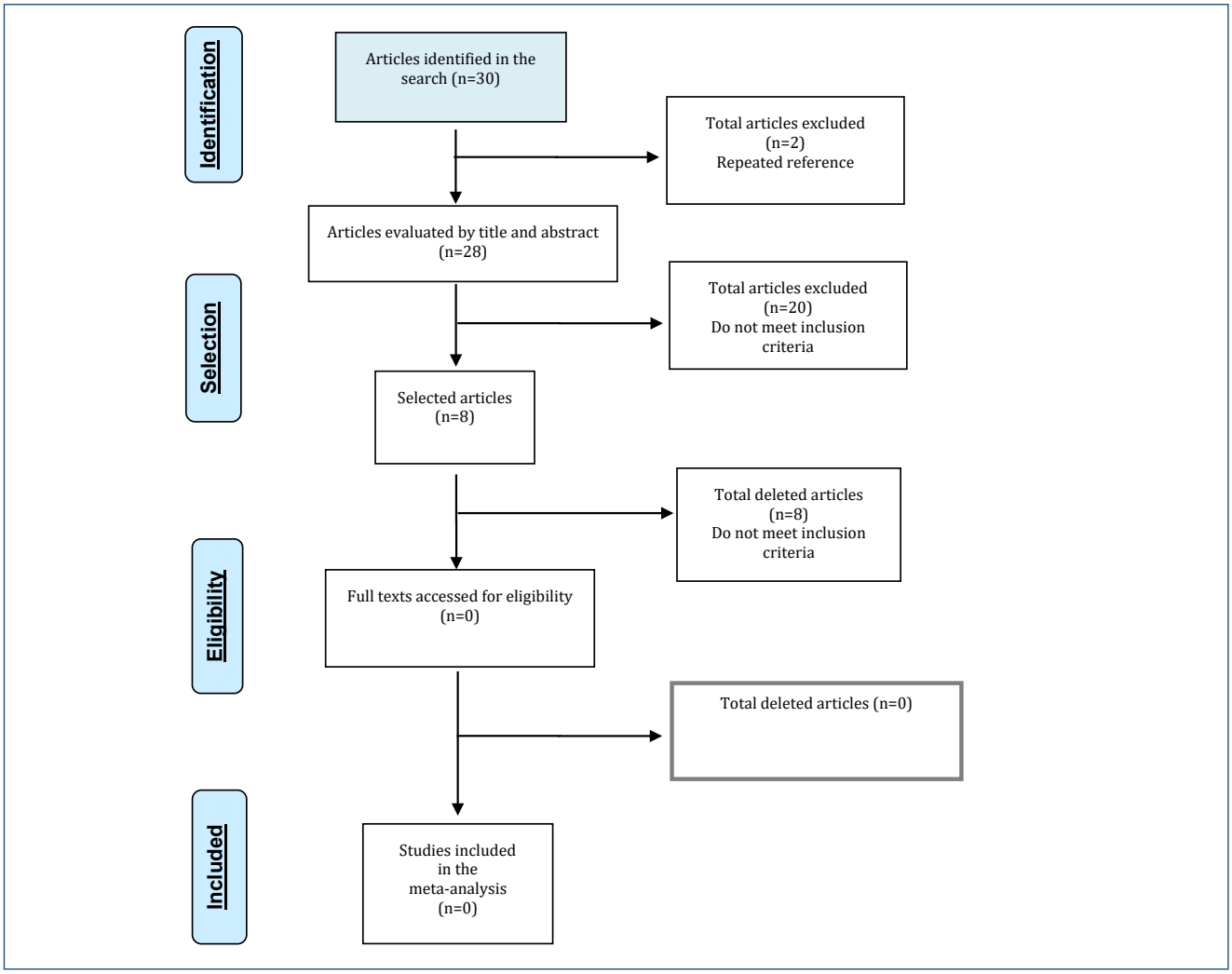

Figure 5. PRISMA flow diagram for regdanvimab.

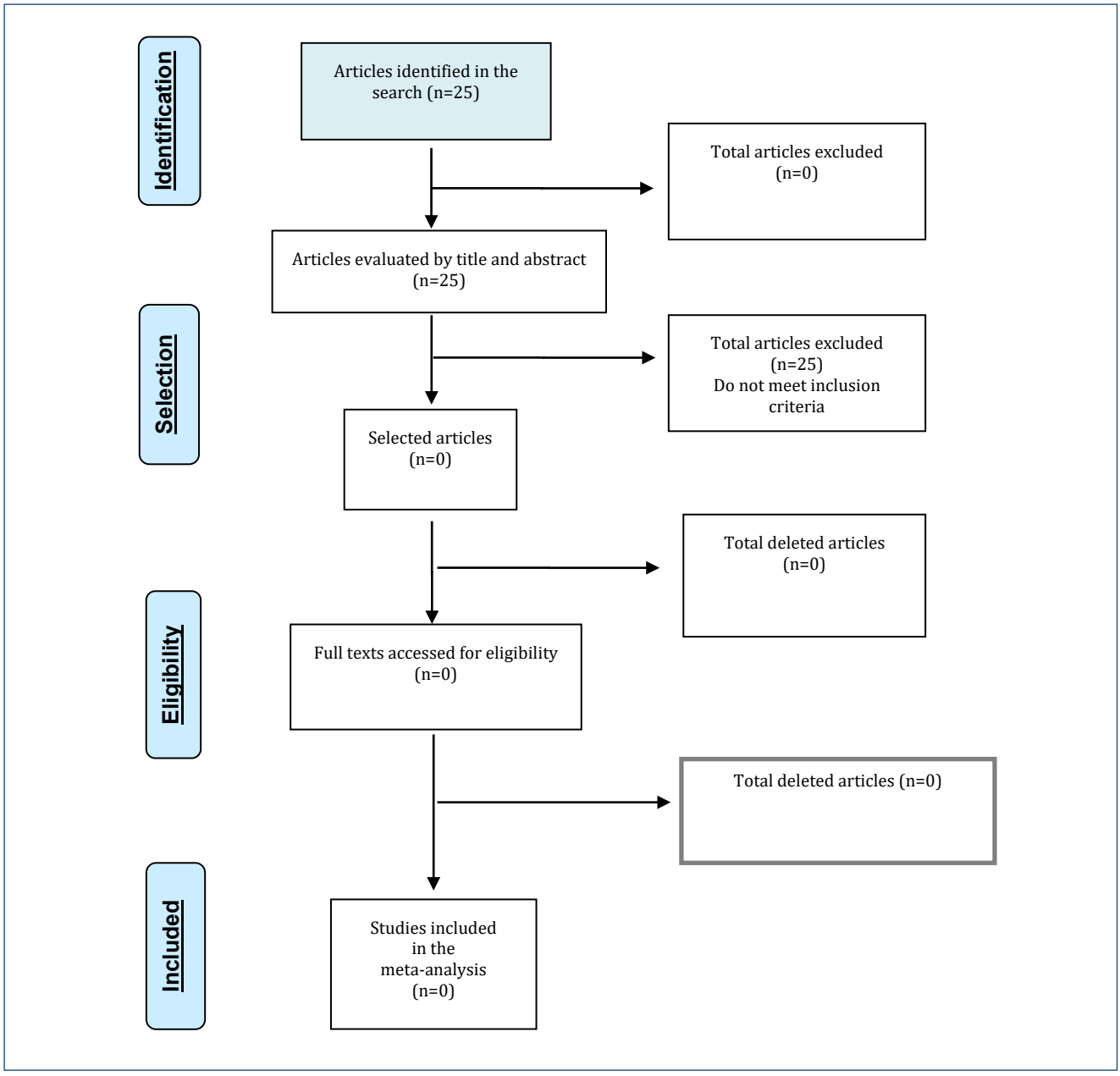

Figure 6. PRISMA flow diagram for tixagevimab+cilgavimab.

- The use of bamlanivimab reduced the hospitalization rate by 4%.
- The use of bamlanivimab+etesevimab reduces mortality risk by 2%.
- The use of sotrovimab reduced hospitalization risk by 6% and 4% of severe adverse event.

In hospitalized COVID-19 patients:

- The use of casirivimab+imdevimab increased the risk of mechanical ventilation or death by 2%.

The quality of evidence to support these recommendations is low.

AUTHORS' CONTRIBUTIONS

SET: Conceptualization, Data curation, Formal Analysis, Writing – original draft, Writing – review & editing. **HB:** Conceptualization, Writing – review & editing. **ANB:** Conceptualization, Writing – review & editing. **WMB:** Conceptualization, Data curation, Formal Analysis, Writing – original draft, Writing – review & editing. **SET:** Data curation, Formal Analysis, Writing – original draft, Writing – review & editing. **DRB:** Data curation, Formal Analysis, Writing – original draft, Writing – review & editing.

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AOP https://doi.org/10.1590/1806-9282.20220190

Role of mean platelet volume in differential diagnosis of adult-onset Still's disease and sepsis

John L Frater[1]*, Cara Lunn Shirai[1]

Dear Editor,

Luo and colleagues have recently published a study entitled "Role of mean platelet volume in differential diagnosis of adult-onset Still's disease and sepsis" in *Revista da Associação Médica Brasileira* examining the role of mean platelet volume (MPV) to distinguish adult-onset Still's disease (AOSD) from sepsis[1]. This is a potentially impactful article, since AOSD can be difficult to distinguish from sepsis and other reactive conditions. In particular, the use of complete blood cell count (CBC) data, which is nearly universally available, can provide a cost-effective means to arrive at the correct diagnosis. The goals of this letter are to (1) compare Luo et al's data to earlier work on this subject and (2) add additional comments regarding the clinical application of MPV.

We followed the guidelines for systematic reviews of diagnostic accuracy studies[2] and PRISMA guidelines[3]. Because this study used publicly available data, it did not require ethical board approval. We conducted an electronic search using Medline (PubMed interface), Scopus, and Web of Science using the keywords "MPV" or "mean platelet volume" AND "Still disease" OR "Still's disease," without restrictions. The date of the search was January 22, 2022. The titles and abstracts were screened, and the full-text articles were then obtained for all potentially relevant studies. After all relevant studies were identified, the reference lists from each paper were reviewed for potentially relevant studies and a search of the PubMed and Google Scholar databases for citations of each paper was conducted to identify additional eligible articles. The included studies were assessed using a standardized data extraction form. The mean and standard deviation (SD) of MPV values were included in a meta-analysis, with the calculation of weighted mean difference (WMD) and its 95% confidence interval (95%CI) in AOSD patients versus patient cohorts with diseases clinically resembling AOSD. For studies where the mean value and SD were not reported, we used the model of Hozo et al.[4] in order to determine the SD from the sample size, median value, and range. Statistical calculations were performed using Meta Mar (www.meta-mar.com, accessed date January 25, 2022). The quality of the included studies was assessed using the Newcastle-Ottawa Scale (NOS)[5], with the articles independently assessed by each author. A final score for each paper was arrived at by consensus.

After a search of the databases, screening of results, and review of reference lists and citations, a total of five studies met selection criteria[1,6-9] (see Table 1). The studies were conducted in China (four studies)[1,6,7,9] and Turkey (one

Table 1. Characteristics of studies analyzing mean platelet volume in adult-onset Still's disease.

Study	Year	Country	AOSD sample size	Control group(s), size and clinical features	Age (years, mean and range)	MPV, AOSD vs. disease control (fL)	Analyzer	NOS
Ge et al.[6]	2021	China	110	84, sepsis	36 (16–74)	9.69±1.31 vs. 10.85±1.24	Sysmex XE 2100	8
Liu et al.[7]	2019	China	82	48, sepsis; 76 healthy	36 (18–74)	9.8 ± 1.2 vs. 11.1 ± 1.1	Sysmex XE 2100	8
Luo et al.[1]	2021	China	68	55, sepsis	33 (18–74)	10.08±1.11 vs. 11.14±1.09	Sysmex XE 2100	6
Ulutas et al.[8]	2021	Turkey	61	61, FMS	39 (19–73)	8.3±1.2 vs. 9.3±1.0	NS	8
Zhang et al.[9]	2020	China	91	89, FUO; 81 healthy	32 (16–74)	9.80±1.23 vs. 10.42±1.03	Sysmex XE 2100	6

MPV: mean platelet volume; AOSD: adult-onset Still's disease; NOS: Newcastle-Ottawa Scale; FMS: fibromyalgia syndrome; NS: not stated; FUO: fever of unknown origin.

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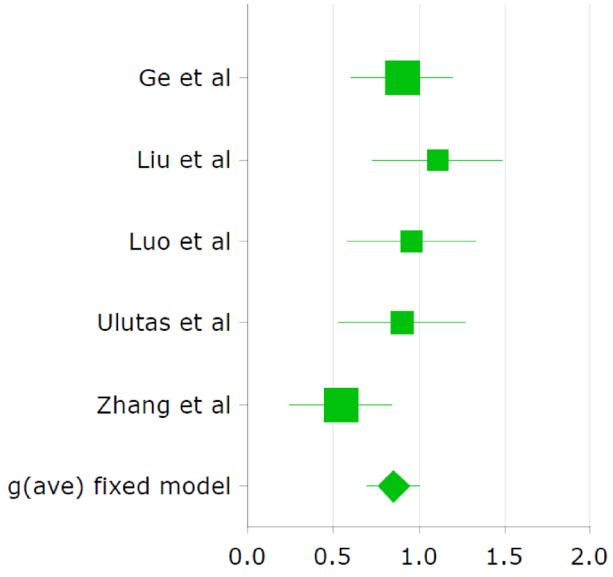
study)[8]. The studies included data on 412 AOSD patients, with sample sizes varying between 61 and 110 subjects. The control populations included 337 patients with clinical features resembling AOSD, including sepsis (3 studies)[1,6,7], fibromyalgia syndrome (1 study)[8], and fever of unknown origin (1 study)[9]. A total of 157 healthy control subjects were included in two studies[7,9]. Four studies used CBCs performed on the Sysmex SE analyzer (Kobe, Japan)[1,6,7,9]; for the remaining study, the blood analyzer was not identified[8]. The standardized mean difference (SMD) for five studies is shown in Figure 1. Since heterogeneity (I^2 statistic) was <50%, a fixed-effects model was used. MPV was found to be significantly higher in disease control patients compared to AOSD patients (SMD=0.85, 95%CI 0.7–1.0). The cumulative data thus support a potential role for MPV in the differential diagnosis of AOSD.

There are several points we would like to emphasize, in particular for clinicians and others interested in the use of MPV for clinical purposes and to assess the MPV as a potential biomarker in AOSD. First, although the quality of the studies was overall adequate or good, with NOS score ranging from 6 to 8, there was overall a lack of information about preanalytical phase variables that could potentially impact results. These variables include time between phlebotomy and analysis, choice of anticoagulant, and storage/transport conditions[10]. Researchers should control for these variables, and clinicians should be aware of these potential sources of bias. In addition, all studies that reported the hematology instruments used in obtaining the MPVs (n=4) used the same instrumentation platform (Sysmex XE, Sysmex). This potentially limits the generalizability of the data since there are continuing problems with cross-platform harmonization of the MPV[11]. Therefore, the reproducibility of these findings has not yet been established when other instrumentation platforms/methodologies are used. In addition, the difference in MPV between the Still's disease and control groups was on



Study	SMD	95%CI Lower	95%CI Upper	Weight (%)-fixed model
Ge et al.[6]	0.90255774	0.60575354	1.199361939	25.69052942
Liu et al.[7]	1.11001512	0.731066673	1.488963566	15.75987892
Luo et al.[1]	0.956677612	0.583757636	1.329597587	16.27353282
Ulutas et al.[8]	0.899687163	0.52938281	1.269991517	16.50423893
Zhang et al.[9]	0.543694439	0.247358704	0.840030173	25.77181991

Figure 1. Standardized mean difference (SMD) and 95% confidence interval (95%CI) of mean platelet volume values in patients with adult-onset Still's disease versus control populations with symptoms suggesting Still's disease.

average ~1 fL. Given the current limitations of the technology and potential problems with preanalytical and analytical variables, it would be difficult to meaningfully interpret individual patient results.

In summary, our analysis adds additional context to the paper by Luo et al that describe the potential role of MPV in AOSD patients. We again thank Luo et al for their addition to this evolving literature and hope that our comments are useful to the readership of *Revista da Associação Médica Brasileira*.

AUTHORS' CONTRIBUTIONS

JLF: Conceptualization, Data curation, Formal Analysis, Methodology, Writing – original draft. **CLS:** Data curation, Formal Analysis, Writing – review & editing.

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A very simple tool to promote asthma education

José Baddini-Martinez[1*], Fernando Sergio Leitão Filho[1], Amanda Maria Reis dos Santos[1], Graciely Matias Guimarães[1], Lilian Serrasqueiro Ballini Caetano[1]

To the Editor,

One of the pillars to achieve asthma control is the degree of knowledge that patients demonstrate about their condition[1-3]. Several studies have shown that greater knowledge about asthma from the patients' perspective is associated with better disease control, fewer acute exacerbations, and improved quality of life[4-6]. Thus, health care providers must take every available opportunity to promote asthma education.

It is usual for patients followed in Brazilian public health services to wait for a significant amount of time in collective areas before their medical consultation. In this scenario, many institutions provide audio and video monitors which continuously show relevant messages and health information. However, due to the considerable costs of such monitors, a substantial number of public clinics and hospitals cannot afford to acquire these pieces of equipment.

To overcome these budget issues and to provide consistent asthma-related information for patients from the Federal University of São Paulo, we created a 30 cm × 43 cm panel containing the following messages in bright-colored letters:

Asthma has no cure! (*Asma não tem cura!*);
But it has control! (*Mas tem controle!*);
Avoid triggers! (*Evite fatores desencadeantes!*);
Use the medication correctly! (*Use a medicação corretamente!*).

This plastic panel was posted in a high and visible place in the collective waiting area of our service. The second panel of the same dimensions was placed right above the first one, showing another message (Asthmatic, check this!; *Asmático, veja isso!*), along with a large arrow pointing to the first panel to draw even more attention from patients.

To investigate the impact of these panels on patient's asthma knowledge, a questionnaire was administered by internal medicine residents and respiratory fellows during the weekly asthma outpatient clinic for 10 weeks before the panels placement and for 11 weeks thereafter. Non-literate subjects or subjects demonstrating cognitive or visual impairment were excluded from the study.

The questionnaire had four questions, displayed in this order:

1. Is asthma curable?;
2. Does asthma have control?
3. Do you think it is important to avoid dust, cats, cleaning supplies, strong smells, and other asthma triggers?
4. Do you think it is important to use the prescribed drugs as instructed during consultations?

For each of these questions, the patients could answer either "Yes," "No," or "I do not know." For statistical purposes, all "I do not know" answers were combined and analyzed together with the "No" answers. After the panels installation, the patients were also asked whether they had seen them and how useful they were from their perspective. Considering that most patients from our clinic are followed every 3 or 6 months, only one notice panel exposure occurred for all subjects.

The Pre-Intervention Group included 82 participants, which consisted mostly of women (n=62, 75.6%), with a mean age of 54.7±17.5 years. For the first question, 37.8% of the responders (n=31) answered incorrectly that asthma can be cured. Regarding the second and third questions, the majority of participants acknowledge that asthma can be adequately controlled (n=78; 95.1%) and that they should avoid, whenever possible, potential asthma triggers (n=77; 93.9%). For the last question, all participants unanimously confirmed the importance of complying with their asthma prescribed medication as instructed during the consultations.

The Post-Intervention Group had 94 subjects (71 were women; 75.5%), with a mean age of 56.8±15.8 years. Of those, 65 (69%) participants admitted they had seen the messages in both panels, whereas 29 (31%) affirmed they had not observed them. When we compared the proportions of expected answers before and after panels placement, we found a significant increase in answers considered correct for the first question

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Table 1. Proportion of observed answers reported by asthmatic patients before and after panels placement containing educational messages about asthma.

	Question 1	Question 2	Question 3	Question 4
Pre-Intervention Group	51/82 (62.2%)	78/82 (95.1%)	77/82 (93.9%)	82/82 (100.0%)
Post-Intervention Group (all)	72/94* (76.6%)	92/94 (97.9%)	87/94 (92.6%)	(94/94) (100.0%)
Post-Intervention (subjects who saw the warning)	51/65* (79.7%)	63/65 (96.9%)	60/65 (92.3%)	65/65 (100.0%)
Post-Intervention (subjects who did not see the warning)	21/29 (72.4%)	29/29 (100.0%)	27/29 (93.1%)	29/29 (100.0%)

*p<0.05 by Fisher's exact test comparing to the pre-intervention group.

based on our entire sample (n=176; p=0.048; Table 1). When considering participants who had seen (n=65) and who had not seen (n=29) the panels, a significant increase in the proportion of correct answers was identified only among the first ones (p=0.047). We did not detect any significant differences between answered and expected answers before and after the panels placement for the remaining three questions.

The current results show that, except for Question 1, the overall answers provided by our patients were quite satisfactory, showing a higher than 90% agreement between theirs and the expected answers, even before the panels placement. These findings are largely explained by the fact that the majority of these participants had been followed in our clinic for several years. In line with this assumption, these results could have been significantly different, if the participants consisted primarily of patients who had been recently admitted to the pulmonology service. Nevertheless, initially, approximately one-third of our participants believed that their asthma was curable or did not know how to answer the question appropriately, which seems to be surprisingly high, since the medical team always reinforce this concept during medical consultations.

The percentage of patients who reported not having seen the panels was 31%. For participants who had seen the panels, 94% of those found the information provided useful. Given that the Pre-intervention group already showed a high percentage of expected answers, we could not identify a clear impact of the panel messages on the accuracy of these questions. However, we did see a significant increase in the proportion of subjects who answered appropriately the first question, with this finding only being observed in the participants who had seen the panels. These results indicate that this simple intervention contributed to a better understanding of our patients regarding their asthma condition. This exposure took place only once for each participant in our study, leading us to assume that recurrent exposures to these panels could lead to even better results. It is also important to emphasize that there was no overlap between participants included in the Pre-and Post-Intervention groups in the study.

In conclusion, asthma-related information, displayed in inexpensive panels in collective waiting areas, can be quite useful in transmitting or reinforcing to patients basic important principles of asthma management. Beyond the scope of this study, we can speculate that this simple educational intervention may help patients to better cope with their asthma and, possibly, achieve a better clinical control.

AUTHORS' CONTRIBUTIONS

JBM: Conceptualization, Data curation, Formal analysis, Methodology, Supervision, Writing – original draft, Writing – review & editing. **FSLF:** Data curation, Writing – review & editing. **AMRS:** Investigation, Writing – review & editing. **GMG:** Investigation, Writing – review & editing. **LSBC:** Investigation, Supervision, Writing – review & editing.

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Comment on "Diagnostic and prognostic significance of long noncoding RNA LINC00173 in patients with melanoma"

Hong Zhu[1], Qian Ma[2], Xianguo Wang[3]*

Dear Editor,

We were very pleased to read the article entitled "Diagnostic and prognostic significance of long noncoding RNA LINC00173 in patients with melanoma" by Wang et al.[1] In this study, the authors revealed that LINC00173 expression was abnormally elevated in melanoma and may serve as a novel biomarker for predicting diagnosis and clinical progression of melanoma patients. However, some concerns need to be raised from our opinion.

The main problem of the study was that it lacks general demographic information, inclusion criteria, and exclusion criteria. There are some factors that affect the prognosis of the melanoma, including size of tumor, status of lymph node, distant metastasis, and complication. Inclusion and exclusion criteria should also be provided. Some chronic diseases such as hypertension and diabetes that affect prognosis should be excluded.

Another concern is that the definition of high expression for LINC00173 was not provided. In this study, 163 melanoma tissues and their pair-matched nontumor specimens were obtained from patients who underwent radical resections at The First People's Hospital of Jinan City from May 2012 to July 2015. LINC00173 was first reported in 2017[2]. Therefore, we can assume that the researchers used frozen samples for the experiment. It is not clear whether freezing storage of general samples would lead to RNA degradation, and whether the researchers considered the effect of freezing time on RNA levels.

AUTHORS' CONTRIBUTIONS

HZ: Data curation, Formal Analysis, Writing – original draft.
QM: Data curation, Formal Analysis, Writing – original draft.
XW: Conceptualization, Writing – review & editing.

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Comment on "Relationship between C-reactive protein/albumin ratio and new-onset atrial fibrillation after coronary artery bypass grafting"

Meilin Ma[1], Lianping He[1], Lingling Zhou[1*]

Dear Editor,

We have read with great interest the article by Fatih Aksoy[1], entitled "Relationship between c-reactive protein/albumin ratio and new-onset atrial fibrillation after coronary artery bypass grafting." The authors find that the novel inflammatory marker C-reactive protein/albumin ratio (CAR) can be used as a reliable marker to predict the development of atrial fibrillation (AF) following coronary artery bypass grafting (CABG). The findings of this survey can bring new judgment indicators for the diagnosis and therapy of AF, to save the diagnosis cost of AF. Nevertheless, we believe that this article still has some issues worthy of further discussion.

The purpose of this study was to compare the predictive value of CAR with other inflammatory markers such as neutrophil/lymphocyte (N/L) ratio and platelet/lymphocyte (P/L) ratio in determining new-onset AF after CABG. Only the prediction accuracy of CAR and CPB was compared in this study. However, the reliability of CAR with N/L and P/L was not explored[2]. Therefore, we recommend that the authors edit the summary objective or add data and statement that can prove the objective. The exclusion criteria of the study included hyperthyroidism, age <18 years, prior cardiac surgery, class III or IV heart failure, previous AF, left atrial diameter >55 mm, left ventricular ejection fraction <0.25, sepsis, heart rate <60 bpm, systolic blood pressure <90 mmHg, inflammatory disease, pericarditis, patients undergoing off-pump surgery, and having antiarrhythmic treatment. The CAR was changed with diabetes, dyslipidemia, and left ventricular ejection fraction[3]. Table 1 reveals that patients with postoperative atrial fibrillation (POAF) were significantly older and more males were affected when compared to patients without POAF ($p<0.001$ and $p=0.003$, respectively) and the presence of diabetes mellitus and hypertension was higher in patients with POAF compared to patients without POAF. Therefore, exclusion criteria should also include obesity, diabetes, or other chronic diseases and the use of drugs that affect C-reactive protein or albumin.

C-reactive protein[4], a type of acute protein, rises sharply in plasma when the body is infected or when the tissue is damaged. The main problem of this study is that C-reactive protein was used to evaluate the degree of tissue inflammation and myocardial function. Heart bypass surgery will inevitably cause certain damage to myocardial function[5], meaning it inevitably enhances the CRP levels. As a result, due to tissue damage, different degrees of albumin levels will decline due to individual differences. Hence, we are suspicious of the practicality of CAR. According to relevant research reports[6], various acute inflammation, tissue damage, myocardial infarction, surgical trauma, radiation damage, and other diseases rapidly increase within a few hours after the onset and have a tendency to increase exponentially. When the *illness* develops, the level of CRP swiftly declines to normal; however, the level of CRP is not always positively correlated with the degree of infection. Level of CRP increases within hours following the operation and then reduces 7–10 days after the operation. If the CRP

Table 1. Characteristics of the subjects included.

	Without AF (n=279)	With AF (n=136)	p
Age (years)	60.5±12.4	67.5±8.9	<0.001
BMI (kg/m^2)	29.0±5.2	28.0±4.4	0.05
Female (n, %)	90 (32.3)	26 (19.1)	0.003
Diabetes mellitus (n, %)	114 (40.9)	73 (53.7)	0.009
Hypertension (n, %)	202 (72.4)	121 (89.0)	<0.001

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does not shrink or rises again, it may be complicated by infection or thromboembolism.

Another point to discuss is that the proportion of the sample (in which 299 are males, accounting for 72%) surveyed by the author is not balanced. There are only 116 females, accounting for 28%. The gender distribution of this study may cause selection bias[7]. In addition, the authors did not provide detailed demographic characteristics such as occupations and residential addresses of the sample, because different occupations or residential addresses have different effects on cardiac function.

DATA AVAILABILITY

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

CONSENT FOR PUBLICATION

All other authors have read the manuscript and have agreed to submit it in its current form for consideration for publication in the *Revista da Associação Médica Brasileira*.

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AUTHORS' CONTRIBUTIONS

MM: Conceptualization, Data curation, Formal Analysis, Methodology, Project administration. **LH**: Conceptualization, Writing – review & editing. **LZ**: Conceptualization, Writing – review & editing.

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Comment on "Diagnosis of long noncoding RNA LINC00173 in patients with melanoma is controversial"

Lingling Zhou[1]*

Dear Editor,

We were very pleased to read the article entitled "Diagnostic and prognostic significance of long noncoding RNA LINC00173 in patients with melanoma" by Wang et al.[1] in which they revealed that LINC00173 expression could serve as an unfavorable prognostic biomarker for melanoma patients. However, some views should be raised in my opinion.

The main problem of the study was that the reliability of conclusions has been questioned in a study published recently. A study found that the LINC00173 was a potential target for the diagnosis, prognosis, and/or treatment of melanoma[2]. However, this article was recently retracted because the authors were unable to provide satisfactory original data for their study[3]. As can be seen in patients and tissue samples section, 163 melanoma tissues and their pair-matched nontumor specimens in this study were obtained from patients who underwent radical resections at The First People's Hospital of Jinan City from May 2012 to July 2015. Nevertheless, LINC00173 was first reported in 2017[4]. It is obviously unreasonable.

In conclusion, due to the above reason, diagnosis of long noncoding RNA LINC00173 in patients with melanoma is controversial.

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Artificial intelligence and machine learning in pediatrics and neonatology healthcare

Felipe Yu Matsushita[1*], Vera Lucia Jornada Krebs[1], Werther Brunow de Carvalho[1]

Medicine has evolved dramatically over the past century. There have been several discoveries, from the invention of antibiotics to the identification of DNA, antipsychotics, and oral rehydration therapy. However, medicine is currently involved by a perplexing paradox: rising spending and worsening health outcomes. Over the past few decades, healthcare has been in the middle of three significant trends: increase in complexity, growing data volumes, and burnout among healthcare professionals. We discussed each of these trends, as well as how artificial intelligence (AI) might aid in the resolution of these issues.

DETERIORATING HEALTH OUTCOMES WITH INCREASING COST

Despite improvements in life expectancy over the past century, mortality trends have remained stagnant in recent decades. In the United States, life expectancy stopped increasing in 2011 and surprisingly began to fall after 2014. Individuals aged 25–64 years have seen an increase in mortality since 2010. The death rates for hypertension and obesity grew by 78.9% and 114%, respectively, between 1999 and 2017. Heart and lung disease, stroke, diabetes, and Alzheimer's disease all contributed to an increase in mortality in early studies[1]. Two reasons why life expectancy stopped improving are the absence of new therapies and higher complexity.

Many clinical disorders still have limited treatment options. The discovery of new therapies and drugs is expensive. Each new medicinal drug was estimated to cost almost a billion dollars in research and development[2]. Moreover, higher costs do not equate to improved essential health outcomes[3]. Pharmacological discovery and development are time-consuming and expensive, with complex processes that can take decades to be approved[4]. Furthermore, the majority of clinical studies for a new drug fail, with efficacy (52%) and safety (24%) being the most common reasons for failure[5].

Moreover, patient heterogeneity cannot be ignored anymore. Medicine in past centuries has focused on developing universal therapies that can treat the maximum number of patients with similar symptoms[6]. However, a wide range of different diseases have similar symptoms, but with distinct mechanisms[7]. It is not a surprise that patients evolve differently, even with the same treatment. Individual variability must be taken into consideration[8]. Precision medicine allows healthcare interventions to be tailored to individuals on the basis of their individuality[4]. Medicine should focus on prevention, personalization, and precision rather than devising therapies for populations and making the same medical decisions on the basis of a few similar physical traits among patients[6]. However, a side effect of evaluating patient heterogeneity is that complexity increases exponentially.

VOLUME OF HEALTHCARE DATA

A physician's ability to examine all healthcare data or stay updated has become impractical. A massive amount of healthcare data is generated every second. Approximately 30% of the world's data volume is created only by the healthcare industry. Data for healthcare will expand at a compound annual growth rate of 36% by 2025, far faster than any other industry[9]. This massive data generation is happening mainly due to the digitalization of healthcare data, high-resolution medical imaging, biosensors with continuous physiologic metrics output, and the OMICS science (genomics, proteomics, metabolomics, and transcriptomics).

The human capacity for analyzing these vast amounts of data has certainly been exceeded. Furthermore, not only the volume of data has increased, but also the variety. Different

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types of health data sources have emerged, such as sensor data, new images techniques, gene arrays, laboratory tests, free text, and demographics[6].

BURNOUT

Burnout is becoming more prevalent among healthcare practitioners. Between 30% and 50% of physicians are thought to be affected. Burnout is linked to lower patient safety, outcomes, and the occurrence of serious medical errors[10]. In addition, physicians with burnout have a higher risk of substance addiction, stress, depression, and suicide[11].

One leading cause of physician burnout is inefficient time management owing to administrative tasks. Without receiving additional incentives, the physician has done an additional 1–2 h of administrative work for every hour spent on patient engagement[11]. An average nurse in the United States spends approximately 25% of her time on regulatory and administrative tasks[12].

The need for providing high-quality care is the first significant component in burnout in healthcare professionals[10]. But how can we deliver high-quality care if we cannot even analyze all the data and spend maximum time on administrative tasks while slowly moving away from the doctor-patient connection, which is (or should be) the heart of medicine? Furthermore, medical error is on the rise in the United States, and it is now the third leading cause of death. It is virtually impossible to completely avoid human error. However, as complexity grows and physicians become overloaded, there is an increase in the number of preventable lethal incidents.

HOW CAN ARTIFICIAL INTELLIGENCE HELP?

Artificial intelligence and machine learning can assist in the resolution of these three fundamental issues by generating new complex insights, increasing computational capacity, and lowering physician workload.

WHAT IS ARTIFICIAL INTELLIGENCE?

Laurence Moroney is an AI lead at Google and explains what AI is in an easy-to-understand manner: A programmer constructs an algorithm by generating a set of rules, expressing them in a programming language, and then using a computer to implement those rules. However, most individuals do not learn a game by being given a set of rules and then blindly obeying them. You play, and then you learn the rules and strategies through experience. So, rather than writing a code that works on the data to get the answers, you will give examples and then let the computer figure out the patterns. They could then turn those patterns into a model that can be used to predict future patterns. In other words, the AI revolution was the idea of using computing power to figure out the rules. Machine learning and big data are already influencing almost every aspect of life. Netflix knows which movies people like to watch and Amazon knows what people want to shop[4].

The AI revolution was only possible because of the increase in computational power. Over time, performance of the computers improved at an exponential rate. Cellphones are currently more powerful than computers were 25 years ago. AI applications can handle massive amounts of data and uncover hidden patterns that would otherwise be lost in the avalanche of wide medical data[13], enabling healthcare professionals to solve complex problems. AI applications are expected to save US$150 billion by 2026 in the US healthcare industry. The shift from a reactive to a proactive healthcare strategy, focused on health management rather than treatment of disease, is responsible for a substantial portion of these cost savings[4]. Also, by reducing mistakes and boosting precision, AI could reduce workload for healthcare personnel while also improving the quality of work provided[14].

EXAMPLES OF ARTIFICIAL INTELLIGENCE APPLICATIONS IN PEDIATRICS

In the past few years, research studies on AI in healthcare skyrocketed. We listed a few examples of applications of machine learning in pediatrics and neonatology.

Machine learning and identification of subphenotypes in extremely low birth weight preterm[7]

Problem

Critically ill patients are the most diverse group in the hospital, with a high prevalence of morbi-mortality. Patients with the same diagnosis often receive the same therapy and strategies, yet their outcomes vary. Patients with various illness mechanisms may be grouped together if they are organized into shallow disorder-based groups.

Population

A total of 215 extremely low birth weight infants who did not have severe congenital malformation.

Artificial intelligence solution

The authors identified six distinct sub-phenotype clusters with different clinical and laboratory characteristics. This means that all preterm infants should not be treated in the same manner.

Machine learning and autism screening in toddlers[15]

Problem

Autism spectrum disorder (ASD) is a neurodevelopmental disorder that affects 1 out of every 59 children. Early detection and management can enhance a patient's prognosis. However, early detection is difficult, and in the United States, the average age of diagnosis is still 4 years.

Population

A total of 16168 toddlers aged between 16 and 30 months.

Artificial intelligence solution

M-CHAT-R scoring was comparable to that of the machine learning system. The M-CHAT-R can be scored objectively and automatically using machine learning.

Artificial intelligence and pediatric head trauma decision rules[16]

Problem

Computed tomography (CT) scanning is the gold standard for quickly diagnosing intracranial damage, but it is expensive, requires sedation, and exposes patients to ionizing radiations. It is quite impossible to avoid unnecessary CT scans.

Population

Between 2004 and 2006, 42,412 children with head trauma and no altered mental status were enrolled in PECARN from 25 emergency departments.

Artificial intelligence solution

Machine learning algorithms may outperform PECARN rules in terms of predictive performance and deliver more tailored and detailed risk estimates.

Machine learning and pediatric sepsis[17]

Problem

In the United States, pediatric sepsis is responsible for about 6500 deaths per year. Early and aggressive treatment of pediatric sepsis is linked to better outcomes.

Population

Children aged 2–17 years, between 2011 and 2016, from a single-center unit, inpatient, and emergency department.

Artificial intelligence solution

Machine learning surpassed the Pediatric Logistic Organ Dysfunction score (PELOD-2) in the prediction of severe sepsis 4 h before the start of the treatment.

Machine learning and neonatal sepsis[18]

Problem

One of the leading causes of morbidity and mortality in neonates is late-onset sepsis. Very preterm infants are more vulnerable, with 10–25% of them developing late-onset sepsis at least once. Antibiotics administered promptly after a diagnosis can significantly reduce mortality, whereas antibiotics administered indiscriminately are counterproductive.

Population

Between 2017 and 2019, 49 preterm (gestational age less than 30 weeks) newborns were admitted to six university NICUs in France.

Artificial intelligence solution

A machine learning system that analyzes heart rate variability in real time (noninvasive) may detect late-onset sepsis with an AUROC of 87.7% as early as 6 h before starting the antibiotics, and with predictive potential (AUROC > 70%) as early as 42 h before starting the antibiotics.

Machine learning and young febrile infants[19]

Problem

Despite the fact that 10% of febrile children aged less than 60 days have serious bacterial infections (SBIs), a considerable majority of those without SBI are categorized as false-positives on the basis of previous decision standards, resulting in wasteful procedures.

Population

A total of 1470 children aged less than 60 days with fever in the emergency department.

Solution

A machine learning algorithm may be able to risk-stratify well-appearing febrile infants aged less than 60 days. This model could have spared 849 (68.5%) of the 1240 individuals who had lumbar punctures.

Machine learning and asthma[20]

Problem

Asthma is the world's most frequent chronic disease among children. It is a multifactorial illness with numerous risk factors. It may be possible to design asthma prevention measures by identifying children who are at a higher risk of acquiring asthma.

Population

A total of 202 children aged between 7 months and 12 years.

Artificial intelligence solution

With an accuracy of 84.9%, a machine learning algorithm could predict asthma in children.

Deep learning and grading hydronephrosis[21]

Problem

Subjective assessment of renal ultrasonography images is used to grade the degree of hydronephrosis.

Population

Children aged 0–116 months with sagittal renal ultrasonography scans were included in the study.

Artificial intelligence solution

The deep learning algorithm correctly graded 94% of the hydronephrosis images.

Machine learning and speech analysis[22]

Problem

Anxiety and depression in children are frequently underdiagnosed. These diseases, if left untreated, are linked to long-term unfavorable effects such as substance abuse and an increased risk of suicide.

Population

Children aged between 3 and 8 years and who spoke English fluently.

Artificial intelligence solution

With an accuracy of 80%, a machine learning analysis of a 3-min speech can be used to detect children with anxiety or depression.

Machine learning and neonatal mortality[23]

Problem

For most poor countries, neonatal mortality is still a major problem. Between 2018 and 2030, an estimated 27.8 million children will die in the 1st month of their birth worldwide.

Population

Between 2012 and 2017, all live births in the Municipality of São Paulo, Brazil (N=1,202,843) were analyzed.

Artificial intelligence solution

Using only normally gathered data, a machine learning algorithm with an AUC of 0.97 could predict the probability of newborn mortality with a very high accuracy.

Machine learning and obesity[24]

Problem

In the United States, childhood obesity is increasing at an alarming rate. Obesity in adults has a number of negative health consequences. Preventing childhood obesity could be essential.

Population

A total of 7519 children aged between 2 and 10 years with at least one BMI percentile recorded.

Artificial intelligence solution

Machine learning system predicted childhood obesity with good accuracy (85%) and sensitivity (90%).

ETHICAL ISSUES INVOLVING ARTIFICIAL INTELLIGENCE

Although we highlighted a few examples of the usefulness of AI in healthcare, it is important to cite some ethical dilemmas.

Artificial intelligence systems will likely make errors in patient diagnosis and treatment. If an AI system makes an

incorrect prediction, who is to be blamed? When it comes to health, this becomes a far more serious ethical issue[6].

Transparency may be the most challenging issue to address with AI. Many AI systems, particularly deep learning image analysis algorithms, are nearly impossible to analyze or explain why the algorithm made a certain prediction. How can we be sure that there are no biases if we cannot explain or interpret the model? Machine learning systems in healthcare may be prone to algorithmic bias, such as predicting a higher risk of disease based on gender or ethnicity when those aspects are not truly relevant[12]. In the context of biomedicine, such systems can strengthen existing sociocultural discriminations that encourage inequities[25].

Artificial intelligence will not replace doctors, as we are dealing with human lives, not simply data. Decisions regarding healthcare are complex and there are many other factors involved, such as communication, doctor-patient relationship, spirituality, and others. However, AI tools will definitely assist healthcare workers with a wide range of duties, namely administrative tasks, clinical documentation, patient outreach, as well as specialized assistance in areas such as image analysis, medical device automation, and patient monitoring.

It is time for pediatricians and neonatologists to embrace AI in order to improve health quality while lowering expenses and administrative workload.

AUTHORS' CONTRIBUTIONS

FYM: Conceptualization, Data curation, Investigation, Methodology, Project administration, Writing – original draft, Writing – review & editing. **VLJK:** Conceptualization, Methodology, Supervision, Writing – review & editing. **WBC:** Conceptualization, Supervision, Writing – review & editing.

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COVID-19 recurrence associated with the virus storage in the Spleen

Andy Petroianu[1]

INTRODUCTION

The spleen is the main fast defense organ due to its capacity of removing foreign particles, old cells, parasites, bacteria, fungi, viruses, and other antigens from the bloodstream without opsonization[1,2]. In general, the particles removed from the blood flow are destroyed and metabolized. However, parasites such as *Plasmodium* and *Leishmania*, viruses such as HIV, and bacteria, after being removed from the bloodstream by the spleen, may not be destroyed and remain within this organ for unlimited time period. Eventually, part of these parasites and viruses leave the spleen and cause transient disease recurrence[1,3-7]. Several studies have shown that after splenectomy, despite reducing the body's defense, the adverse events of these diseases disappear or reduce their intensity[1,5,6]. Most people who acquire COVID-19 persist asymptomatic, indicating that human defenses are able to control this virus. However, some people who had manifested this disease, and despite having been vaccinated, may present new episodes of COVID-19, which are ascribed to a new contamination[8,9].

According to these findings, our point of view is that SARS-CoV-2 can be removed from the bloodstream by the spleen and stored without being destroyed. Eventually, this virus recirculates and reactivates the disease without a new contagion. This possibility can be experimentally assessed in several mammals and also in human clinical trial.

METHODS

To verify the reinfection by coronavirus stored in the spleen, experimental and clinical studies may be performed as follows.

Experimental study

This proposal must be submitted to an ethics committee for research in animals and started only after its approval.

The Coronaviridae family has been investigated in many animal models, such as normal and genetically modified mice, rats, hamsters, rabbits, and pigs, among others[10-16]. Due to the high number of animals needed in this study, rabbits seem to be the most appropriate[10,11,13]. A non-lethal coronavirus (CoV) associated with mild disease is required to preserve the animals alive during all these experiments. According to literature data, the MERS (*Middle East respiratory syndrome*) beta-CoV seems to be the most adequate for this infection[10,13,14].

A total of 60 rabbits will be contaminated with intranasal inoculation of 10^3 $TCID_{50}$ (median tissue culture infectious dose) of EMC/2012 MERS-CoV strain[10,13,14]. These animals will be distributed in six groups (n=10) with equal number of males and females (n=5), according to the follow-up period as follows: group 1 (7 days), group 2 (14 days), group 3 (21 days), group 4 (30 days), group 5 (60 days), and group 6 (90 days).

At the end of the follow-up period, under general anesthesia (ketamine 10 mg/kg and propofol 2 mg/kg intravenous injection), the spleen will be removed after the ligature of its vascular pedicle through a median laparotomy. Spleen samples will be processed in the routine pathological analysis and stained with hematoxylin and eosin for microscopic study of histopathological findings caused by the CoV. Other spleen specimens will be processed in the real-time quantitative *polymerase chain reaction (RT-qPCR)* assays for the detection of the MERS-CoV by using appropriate primers kits[10,13,14]. The results of all groups will be

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statistically compared to verify the intensity and length of the spleen damage. The presence of the MERS-CoV in splenic parenchyma will be assessed as well, with emphasis on its intensity and period of its occurrence.

CLINICAL STUDY

This project must be submitted to an ethics committee for research in humans and only started after its approval.

This study will be a multicenter trial and carried out using electronic means of sections linked to the registry of patients who have been diagnosed with COVID-19. All patients who had recorded recurrence of their disease regardless of age and sex will be included and investigated. These patients will be contacted by telephone, social network, email, or personally. The researchers will introduce themselves and explain the purpose of this study. They will then request the consent of patients to be included in this work.

After signing the consent, each volunteer will be identified by age, gender, occupation, and ethnicity. They will be asked if they have undergone a surgical procedure, highlighting the splenectomy and the disorder that indicated this procedure. The date of splenectomy and the date of infections by COVID-19 and of its recurrences will be recorded. These patients will be divided into two groups, with and without spleen, to statistically compare the incidence of COVID-19 recurrence in both the groups.

DISCUSSION

The animal study may verify the presence of the CoV stored inside the spleen even after the animals be considered healthy and without signs of any disorder. Even being apparently cured, the alive virus can be present in a spleen that was not able to destroy it. Thus, the virus may multiply in the spleen and return to the bloodstream as a recurrence of the disease without a new contagion[1,4].

The multicenter clinical trial may assess the recurrence of COVID-19 in splenectomized volunteers. If the hypothesis of this article is correct and no recurrence will be found in splenectomized patients, further studies will be necessary to characterize whether the recurrence is due to a new contagion or due to the persistence of live COVID-19 in the spleen. This knowledge will be useful to better understand the infection caused by COVID-19 and to recommend more specific propedeutics and therapeutic strategies. Despite the possible presence of COVID-19 stored in the spleen, it is worth emphasizing the importance of keeping this organ due to its multiple relevant functions, including the vaccine efficacy[1].

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ORIGINAL ARTICLE

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Cadaveric study of anatomical measurement of isthmus parameters of lumbar spine to guide cortical bone screw placement

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SUMMARY

OBJECTIVE: To reduce surgical exposure and improve accuracy, this study evaluated the anatomical distance parameter D (including D1, D2, and D3) of the lumbar isthmus for cortical bone screw insertion.

METHODS: A total of 25 structurally complete lumbar dry specimens were used for lumbar anatomy measurements. The six cadaver specimens were divided into upper and lower parts on the plane of the T11–T12 vertebrae, and we use the lower parts. Therefore, six lumbar wet specimens and another four complete lumbar dry specimens were selected. The lumbar isthmus tangent point was considered a coordinate origin, and the insertion point was determined through translating the distance of D1 value to the midline of the vertebral body horizontally and then vertically moved toward inferior board of the transverse process with the distance of D3 value.

RESULTS: In four dry and six wet intact lumbar specimens, cortical bone screws were placed according to the average value of the isthmus parameter D. A total of 100 trajectories were verified in specimens by X-ray and computed topography scan to evaluate the safety, accuracy, and feasibility of the surgical use of isthmus parameter D. Using this parameter, the rates of excellent screw placement were 95% (38/40) in four dry specimens and 88.7% (53/60) in six wet specimens.

CONCLUSION: The isthmus parameter D is easier to use by the operator, which can improve surgical accuracy and reduce operation time.

LEVEL OF EVIDENCE: Level IV, prospective study.

KEYWORDS: Lumbar. Anatomy. Cortical bone trajectory (CBT).

INTRODUCTION

The concept of cortical bone trajectory (CBT), proposed in 2009[1], is a new screw placement method that changes how the pedicle long axis is used as a trajectory for traditional pedicle screws. CBT makes trajectories display partial deviation on the head in the sagittal plane and for the outward angle on the cross section, ensuring that the screw is fitted with the cortical bone of the lateral edge of the pedicle and the upper end plate of the lumbar vertebra[2,3]. Compared with traditional pedicle screw technology, CBT has become an ideal internal fixation method for patients with osteoporosis and revision surgery[4,5].

Previous studies considered the ideal screw insertion point for lumbar cortical screws to be coronal at the intersection of the vertical midline of the articular process with the 1 mm horizontal line below the transverse process of the same side[1,6-8]. However, the reference for this method was based on mild degenerative lumbar facet joints and could not be localized for cases with serious facet joint hyperplasia.

In the present study, the isthmus parameter D was employed to determine CBT screw placement. Specimen measurement and screw insertion of anatomical samples were performed to investigate the accuracy and clinical safety of the use of isthmus parameter D as the new cortical bone screw insertion reference point.

METHODS

Object selection

A total of 25 structurally complete lumbar dry specimens were used for lumbar anatomy measurements. Another four complete lumbar dry specimens and six lumbar wet specimens were selected for screw insertion and CBT evaluation. This study was

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also approved by the Ethics Committee of the First Clinical Medical Institute of Xinjiang Medical University (approval no.: 20141218-01).

Anatomical specimen measurement

Based on our previous studies of traditional pedicle screw placement[9] and lumbar isthmus parameter measurement[10], the isthmus parameter D1 could not be directly measured on anatomical specimen, but could be derived from S1 and S2. Because it is not easy to put the arm of vernier caliper into the spinal canal of the anatomical specimen when measuring the distance S2 between the inner wall of the pedicle, the fine Kirschner measure method was adopted to accurately measure the distance S2 in the present study[9]. To better confirm the measurement base point, we selected the distance between the inner wall of the pedicle and the vertebral body junction (point) on both sides. After removing the specimen, we used a vernier caliper to measure the distance between the two fine Kirschner pins to get the distance S2. The straight distance between the tangent point of the lateral edge of the isthmus and the tangential line of the pedicle inner wall (parameter D1) was obtained from the formula: D1=(S1–S2)/2.

The lumbar vertebrae were fixed on a foam plastic board with Kirschner wire and adjusted until the vertebral body was completely perpendicular to the board surface. D2 was measured as the vertical distance between the line connecting the vertexes of the isthmus and the lower edge of the transverse process. We separately measured the left and right D2 values and recorded the average as the final D2 value (Figure 1). If the lower edge of the transverse process was not a straight line, we applied the short distance between the end of the accessory process crest and the base of the transverse process, as the baseline of D2 measurement, and marked it on the specimen.

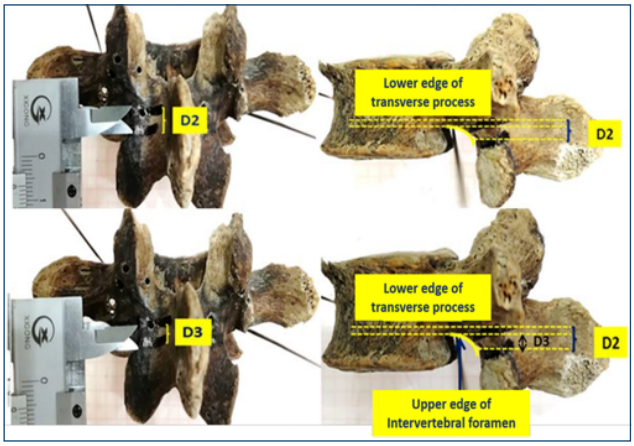

Figure 1. Diagram of the measurement of isthmus parameters D2 and D3.

Anatomical specimen lumbar isthmus parameter D3 obtained by the calculation "D2–1 mm" was the vertical distance from the line connecting the tangent points to 1 mm below the lower edge of the transverse process (Figure 1).

Screw insertion point selection

Without changing the horizontal axis, the vertical axis of the insertion point of the cortical bone screw was moved from the conventional mid-perpendicular line of the articular process to the tangent line of the median wall of the pedicle[8,10]. The lumbar isthmus tangent point was taken as a benchmark and then the D3 value was longitudinally shifted toward the cephalic side to obtain the vertical axis of the cortical screw insertion point. The horizontal axis of the screw insertion point was confirmed by referring to the D1 value of the lateral edge of the isthmus. The point where the vertical and horizontal axes intersected at the inside edge of the isthmus was regarded as the cortical screw insertion point. To enhance the holding power between the proximal cortex and the cortical bone of the lamina and pedicle, we used modified CBT screws that are different from traditional pedicle screws[1].

Anatomical specimen screw insertion

The screw insertion points on the 4 complete lumbar dry specimens and 6 wet specimens were based on the average values of isthmus parameters D1, D2, and D3 measured on 25 dry samples. All 100 trajectories were evaluated through visual observation, probe penetration, X-ray, and CT scans. The evaluation criterion was scored as described previously[11].

Statistical analysis

All analyses were performed using SPSS version 18.0 software (SPSS Inc., Chicago, IL, USA), and the results are presented as mean±standard deviation (SD). Variance analysis was used for comparisons between internal subgroups.

RESULTS

Anatomical specimen isthmus parameters

D1 gradually increased from the upper to lower lumbar spine (Table 1). The safety range of D1 should be limited between 2.5 and 5.5 mm. The D1 values of male patients were larger than those of female patients. Notably, D1 in the L5 segment should never exceed 6 mm to prevent the screw from entering the spinal canal during the insertion process.

The results of anatomical specimen distance D2 are presented in Table 1. The isthmus parameter D3 was defined as

D2–1 mm, indicating that D3 had a pattern similar to D2 (Table 1). D3 was based on the tangency point of the side edge curve of the isthmus. Compared with the traditional reference of the transverse process, the left and right sides of the isthmus were more symmetrical, suggesting that using D3 as a reference could improve screw insertion accuracy.

X-ray and CT examination results

The excellent rates of imaging evaluation results were 88.7% (53/60) and 95% (38/40). According to the previously published criterion[11], in wet lumbar specimens, four trajectories were evaluated as Grade II and three trajectories were considered as Grade III. For four dry specimens, two trajectories were evaluated as Grade II. These values demonstrated that the cortical screw insertion method based on isthmus parameter D was safe, accurate, and feasible.

DISCUSSION

Cortical bone does not undergo significant deformation and degeneration with age, so even osteoporotic cortical bone is relatively intact, although cancellous bone undergoes significant degeneration and has seriously reduced intensity[12]. Zdeblick et al. pointed out that the torque at screw insertion into the bone is the best indicator for predicting the failure of the bone and screw interface; that is, the bone strength determines whether the screw is loose[13].

From an anatomical perspective, to increase the holding force, the cortical bone screw insertion point should be as close as possible to the inner wall of the pedicle. To avoid damaging nerve structures within the spinal canal[14], the insertion point of the screw head should be proposed to the tangential site of the medial wall of the pedicle at the lateral edge of the isthmus. Using the modified CBT technique, the thicker periphery of the cortical bone screw at the insertion point (especially the lateral edge of the cortex) increases screw strength and avoids spondylolysis due to screw displacement. In addition, moving the cortical bone screw insertion point to the median side of the lumbar spine can also reduce the impact of the screw tail on the facet joint by increasing the distance between them; this reduces further degeneration of the facet joint.

Measuring isthmus parameters is helpful to estimate D values (including D1, D2, and D3) during surgery. A previous study proposed using X-ray to confirm the position of the highest intervertebral foramen point to further determine the optimal insertion point[16]. The tail of the lumbar accessory process crest was nearly consistent with the level of the lower edge of the transverse process. The distance between the accessory process near the base of the lumbar transverse process can be a good reference if it is difficult to identify the lower edge of the transverse process. This method reduces exposure of the transverse process and paraspinal muscles, minimizes soft-tissue injury and bleeding, and shortens operating time.

Notably, the most important factor affecting insertion torque was the length of the cortical screw in the lamina, not the length in the vertebral body or the total length of the screw[15]. Further measurements showed that the thickness of

Table 1. Distances D1, D2, and D3 measured by Vernier calipers on human lumbar spine specimens.

Lumbar segments	D1 (χ±s mm)	D2 (χ±s mm)		D3 (χ±s mm)	
L1	1.92±0.12	Left side	4.83±0.87	Left side	3.83±0.87
		Right side	4.84±0.85	Right side	3.84±0.85
		Average value	4.84±0.86	Average value	3.84±0.86
L2	2.06±0.09	Left side	5.98±0.77	Left side	4.98±0.77
		Right side	5.97±0.78	Right side	4.97±0.78
		Average value	5.98±0.77	Average value	4.98±0.77
L3	3.36±0.24	Left side	5.26±0.84	Left side	4.26±0.84
		Right side	5.25±0.84	Right side	4.25±0.84
		Average value	5.26±0.84	Average value	4.26±0.84
L4	4.38±0.15	Left side	3.75±0.41	Left side	2.75±0.41
		Right side	3.77±0.41	Right side	2.77±0.41
		Average value	3.76±0.40	Average value	2.76±0.40
L5	5.54±0.24	Left side	2.22±0.37	Left side	1.22±0.37
		Right side	2.19±0.36	Right side	1.19±0.36
		Average value	2.20±0.37	Average value	1.20±0.37

the isthmus or lamina at the cortical screw insertion point gradually increased. The L1 isthmus was 7 mm, and the value increased from L2, up to 10.5 mm at L5. According to the geometric angle, when the thickness of each segment of the lumbar spine was fixed, the effective length of the screw in the lamina could only be increased by enhancing the abduction angle of the cortical bone screw. CBT technique could increase the length of the cortical bone thread by at least 5 mm, or at least two full turns, which improves screw stability in the lumbar spine.

These values are similar to the excellent rates reported for traditional cortical bone screw technology in the literature[17,18]. These results demonstrated that the cortical bone screw placement method based on isthmus parameter D was safe, accurate, and practical. During CBT screw insertion, exposing the lateral edge of the isthmus is sufficient for screw placement without additional exposure of the transverse process. Screw insertion at the intersection of the two isthmus parameters (D1 and D3) will make surgery safer, less invasive, and easier. It will reduce intraoperative bleeding, fluoroscopy location time. We recommended that the screw placement angle for improved CBT technology should be larger than the recommended abduction angle of 10° for traditional CBT screw placement. The L1 and L2 angles in the upper lumbar spine should be controlled at approximately 10°, the angle of L3 should be 10°–15°, and those of L4 and L5 should be 15°–20°.

Furthermore, our modified cortical screw placement method did not change the horizontal axis of the cortical screw placement coordinate system; rather, it shifted the placement point further toward the midline, increasing the thickness of the peripheral cortical bone of the screw to increase the initial stability of the screw placement and the holding power (Figure 2). The tail of the screw is not near the intervertebral space level or corresponding articular joints, so it does not affect interbody cage placement in clinical practice. In special cases that require a very large cage, the screw is placed in the previously prepared screw path after cage placement. However, because the screw insertion point of this modified CBT technology is closer to the spinal process and laminar decompression area, there are certain limitations of application for patients requiring extensive lamina decompression. Thus, we recommend application of traditional pedicle screws in such severe clinical cases, and novel CBT was adopted for patients with mild or moderate lumbar spinal stenosis.

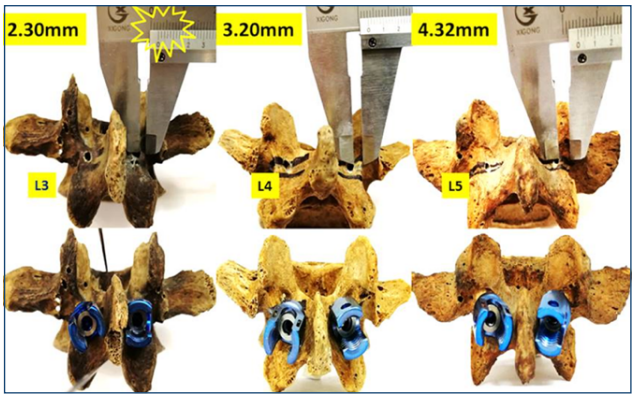

Figure 2. The insertion point of L3–L5 lumbar vertebrae using the modified cortical screw technique.

CONCLUSION

The isthmus parameter D is easier to use by the operator, which can improve surgical accuracy and reduce operation time.

AUTHORS' CONTRIBUTIONS

PR: Conceptualization, Methodology, Writing – original draft. **DA:** Software, Formal Analysis. **SW:** Visualization, Validation. **NA:** Resources, Investigation. **AK:** Data curation. **SW:** Conceptualization, Methodology, Supervision. All authors read and approved the final manuscript.

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ORIGINAL ARTICLE

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Knowledge of gynecologists in the public health system care of women victims of violence

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SUMMARY

OBJECTIVE: This study aimed to evaluate the knowledge of the obstetricians and gynecologists in the care of women victims of violence in the public health system and the existence of institutional mechanisms to support them.

METHODS: A cross-sectional and observational study was conducted with an electronic questionnaire by physicians who provided care in the obstetrics and gynecology emergency unit of the public health system. This study aimed to identify the care for victims of violence who received the institutional mechanisms of support, the difficulties encountered in determining the appropriate care, and estimates of the prevalence of violence against women.

RESULTS: Notably, 92 physicians responded to the questionnaire. Of these, 85% had already provided care in one or more cases of violence, and 60% believed that <20% of the women received adequate care in these cases, mainly due to the short-time frame of the consultation, lack of team preparation, and lack of institutional resources. A total of 61% of the participants believed that they were not prepared to provide adequate care in those cases.

CONCLUSIONS: Most of the physicians interviewed, although reported to have sufficient knowledge to adequately treat victims of violence, did not provide such care due to lack of institutional support.

KEYWORDS: Gender-based violence. Intimate partner violence. Sex offenses. Domestic violence. Patient-centered care. Obstetrics and gynecology department, hospital.

INTRODUCTION

Sexual, domestic, and intimate partner violence against women is a public concern worldwide, because one in every three women will face violence in their lifetime[1,2]. In 2016, 4,645 Brazilian women lost their lives due to violence of this kind, which has grown by 6.4% in the past 10 years[2,3].

The concept of violence against women may be understood as a relationship characterized by power inequality, which is the end result of a historical process and sociocultural subordination of women to men[4]. Its specific characteristics include male offenders (in 70–90% of cases worldwide), an intimate partner, someone who is trusted, and a family or affectionate relationship linked to the victim and, most of the time, the violence occurs in the domestic environment[2].

Besides being a violation of human rights, violence against women causes immediate and long-term health outcomes, including physical trauma, unwanted pregnancy, miscarriage, gynecological complications, the transmission of infectious diseases and mental disorders[5,6], and high-risk factors for developing a smoking habit or alcohol and drug addiction[7]. Prevention strategies are still scarce and only a few, such as scholastic education, micro-funding programs for women, and reduction of access to alcohol, are efficient but still not widespread[8].

In Brazil, the Maria da Penha Law (Law No. 11.340/2006) was passed in order to treat the phenomenon of domestic violence in an integrated manner[9]. Under this law, social assistance instruments were created to provide victims with lifestyle alternatives, protection, and emergency care[10]. The health system constitutes one of the main gateways of support for these women, because in many cases they seek out these services before calling the police or resorting to special courts for relief[11,12].

Since 2011, declaration No. 104 of the Ministry of Health established the requirement for compulsory notification of any identified or reported case of domestic or sexual violence[9].

Health professionals, however, face a number of difficulties and limitations in the notification of cases, such as how to recognize victims, how to approach and screen

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patients, and even more difficult, how to manage them in the hospital[13,14]. In a study conducted by the Royal College of Physicians and Surgeons of Canada, 94% of gynecologists (GYN) never had a protocol for screening such victims and the same percentage of participants believed that the screening was inadequate[15].

National and international studies in bioethics point out still greater barriers to delivering this physician-centered care, which includes value judgments about victims, lack of training of professionals, and the invisibility of cases in which there was no active questioning[16,17].

The gynecologist is often the first responder providing care for these victims, and the studies conducted so far have shown a lack of knowledge on the part of GYN and obstetricians (OB) regarding the identification of cases and their management[18].

The objective of this study was to evaluate the knowledge level of OB/GYN concerning the provision of care to women victims of violence in the public health system and to ascertain the existence of institutional mechanisms in order to support victims and provide adequate care.

METHODS

Design of the study and study population

A cross-sectional and descriptive study was conducted with an electronic questionnaire (Appendix 1) by obstetrics and gynecology physicians who provided emergency care in the public health system irrespective of the hospital.

Participants were recruited through suitable WhatsApp groups. The transmission list was composed only of OB/GYN who worked in a gynecological emergency room and included 860 professionals from more than nine different hospitals in the state of São Paulo, Brazil.

An electronic invitation was sent to the groups who agreed to participate in the study. At the beginning of the questionnaire, we applied the inclusion criteria and, after data collection, the exclusion criteria for the study.

1) Inclusion criteria: physicians working in the OB/GYN emergency room at any hospital in the public health system, regardless of whether they also delivered care in the private sector, who agreed to and signed the informed consent form.
2) Exclusion criteria: physicians who delivered outpatient care only in a private hospital and did not either agree with the terms of the informed consent or complete the electronic questionnaire.

A self-applied electronic questionnaire required participating physicians to complete all 19 questions about the care of women with complaints or signs of violence (physical, domestic/intimate sexual partners) in the OB/GYN emergency room at public health hospitals.

The authors created questions based on a questionnaire completed by physicians working in prenatal care in the study by Alicia J. Long et al.[15] that was adapted for daily care in the emergency room. The authors included questions on the difficulties encountered by health professionals when providing care to victims of violence, as pointed out in other studies published in the literature[16], and the definition of domestic violence according to the Brazilian law[19].

The questionnaire (Appendix 1) was validated by means of a pilot study, including eight physicians. At the end of the questionnaire, an open field was added to record suggestions and critiques to be used in the development of the final questionnaire.

Ethical aspects

This project was approved by the Ethics Committee for Research Involving Humans, CAAE no. 14503519.7.0000.0071. The informed consent form was made available on the first page of the electronic questionnaire, and the confidentiality of the participants who responded to the questionnaire was strictly observed.

Statistics

No formal calculation of sample size was performed due to the largely descriptive nature of the research. Convenience sampling was used to select the number of participants recruited.

Categorical variables were described by their absolute and relative frequencies and numerical variables, using means and quartiles, in addition to minimum and maximum values[20]. To investigate possible associations between questions and categorical responses, the chi-squared or Fisher´s exact test was used, and the groups of interest were compared via estimates of percentages using the Mann-Whitney non-parametric tests. Analyses were conducted with the aid of the statistics package, SPSS, and the significance level used was 5%[20].

RESULTS

Of the 860 physicians who received an invitation to participate in the research (excluding those who simultaneously participated in more than one group), 104 responded to the questionnaire and 12 met the exclusion criteria. Therefore, the final sample for this study included 92 responses that were analyzed.

The majority of participants were women (83.7%), and 71.7% of the questionnaires were completed by physicians aged up to 30 years. Of these, 69.6% of participants also provided care in the private health sector.

Among the included professionals, 89.1% had already assisted in a case of violence against women, but 30.4% of these physicians reported the absence of any protocol for the care of these victims at their working institution. In addition, concerning programs at the hospital on awareness and/or training of professionals to identify and assist these patients, 44.6% responded that these did not exist, and 37% did not know whether this type of program was present or absent at their institution. Of the 18.5% who responded "yes" to this question, 53% have never participated in such programs.

Concerning their experience with the violence situation, 47 of the 92 professionals had already experienced such a situation in their proximity. More than 90% of the professionals estimated that ≥20% of all adult women had suffered domestic violence at any time in their life.

Signs that drew the professionals' attention to the possibility that a patient was the victim of violence were as follows: symptoms or physical signs of aggression (92.4%), a medical history incompatible with the observed symptoms (88%), reports of having "problems at home or with partners" (82.6%), reports of having already suffered violence in the past (73.9%), patients who introverted, quiet, or distant during the consultation (69.6%), patients who cried (59.8%), and patients who reported an addiction such as drugs, alcohol, or tobacco (41.3%).

In relation to the approach taken to the patient, 80.4% of the professionals reported receiving important help from the multidisciplinary team. Only 59.8% reported that they talked to the patient about the importance of seeking help from family and friends. Only 4 reported not to broach the subject of violence. Of note, 63% of the professionals discussed the subject with the patient, 16% discussed the subject only if the patient reported violence, and 19.5% discussed the subject only to rule out a serious condition.

On case management measures to be taken, more than 80% of the professionals mentioned privacy guarantees and establishment of an environment of care, contraception and disease prevention, referral to a violence service after the initial care, activation of institutional protocol (existing cases), detailed and complete medical record, and care in coordination with the multidisciplinary team.

A total of 94.6% of these professionals believed that less than half of the patients who were victims of violence received adequate care in the OB/GYN emergency unit. The main difficulties mentioned by at least half of them were unprepared multidisciplinary team; short-time frame of visit (more than 70% of them reported a period of 10–15 min); lack of resources; and lack of knowledge of the resources available, what questions to ask, or how to express themselves. A total of 27.2% of the respondents reported feeling uncomfortable while discussing the subject.

Among the general questions about the care delivered, 58.7% believed that they were not providing adequate care to the victims of violence. Among those who believed that the care was adequate, 46.7% pointed to institutional support as the main contributing factor. All professionals responded that they would value the existence of an institutional protocol for violence cases and that they would be likely to follow it.

In a comparative analysis of the questions, we could discern that the existence of institutional protocols led professionals to feel more confident in care delivery. Although there was a tendency to improve the quality of care among health professionals who worked at institutions where there are protocols or who had participated in training and awareness-building activities, no statistically significant association was found.

Half of the professionals, who worked at institutions where there is a protocol, believed that they are providing better care for patients, compared to only 26.5% of the professionals at hospitals where there are no protocols or where there is only a referral protocol, and the difference was statistically significant (p=0.027) (Figure 1).

The percentage of professionals who believed they are providing adequate care to victims of violence was 75.0%; out of those who participated in training/awareness-building activities were promoted by their institution, and this perception was significantly higher than in groups who worked at institutions that lacked such activity or who have never participated in them (38.1%), although there are no statistically significant differences between these factors (p=0.061) (Figure 2).

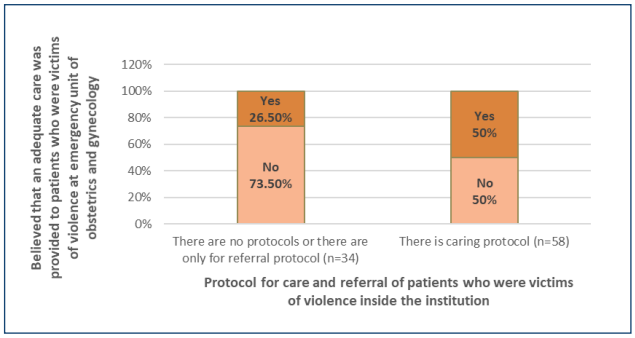

Figure 1. Relationship between institutional protocol and the belief that the care provided by physicians who participated in the study is adequate to patients who were victims of violence.

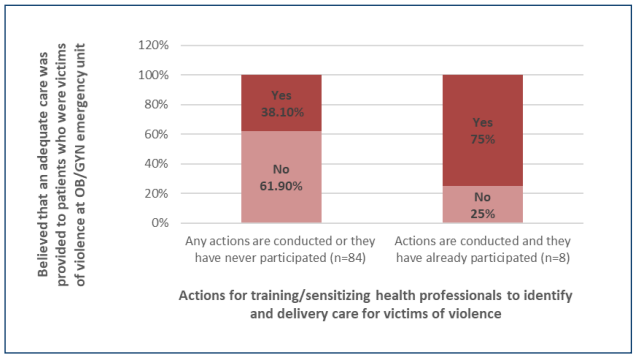

Figure 2. Relationship between institutional activities and adequate care delivery by physicians who participated in the study to patients who were victims of violence.

DISCUSSION

Our main findings in this study were that OB/GYN know how to furnish adequate care to victims of violence; however, we suggest that the main barrier to guarantee such care is a lack of institutional support as well as a standardized care protocol.

Approximately 90% of the respondents had already provided care in a case of violence against women and believed that 20% or more of the women population had already suffered some form of violence. Such a perception agrees with data from the World Health Organization (WHO)[16,17], which confirmed the relevance of cases of domestic violence in our society.

The main signs that drew the attention of the professionals to the possibility that patients were victims of violence are in agreement with those reported in the literature, namely, symptoms or physical manifestations of aggression, a medical history incompatible with the observed symptoms, reports of having "problems at home or with a partner," and reports of previous addictions, and all these situations are compatible with the risk factors identified in the WHO Handbook of Injury and Violence Prevention[7].

However, our sample of physicians apparently showed greater knowledge compared to studies in the literature. The study of Souza, a review of 16 articles on the care of violence victims, showed that 8 of them pointed to the lack of adequate training in medical education as one of the main barriers to adequate care[16]. Lack of knowledge was also described in a Canadian study that evaluated prenatal care physicians[15].

Concerning their approach to the patients, respondents were concerned about seeking help from the multidisciplinary team and about family support, fundamental points proposed in the protocols of the Ministry of Health and Federation of Gynecology in the State of São Paulo[18,21]. Only four physicians reported that they limited themselves to the patient's main complaint and did not investigate the suspected violence, data repeatedly reported in Hasse and Vieira's articles , which showed that 8.2% of the physicians had an inadequate approach[22].

Almost all interviewed professionals (94.6%) believed that less than half of these patients received adequate care in the OB/GYN emergency. An Australian study that addressed violence by an intimate partner in the setting of emergency services also pointed out that these difficulties are associated with the challenge of maintaining a non-judgmental posture and not mixing up care with their own sentiments, particularly among professionals who have experienced violence previously[23].

Of the interviewees, 30.4% responded that the place where they worked lacked any protocol for the care of the victims of violence; however, all professionals would be likely to follow an institutional protocol for care and referral of these patients. The WHO data confirm the importance of existing institutional protocols in facilitating and enabling adequate care of the victims of violence, where the direct approach and referral procedures for the case are identified[7].

It is of fundamental importance that OB/GYN provide an environment that allows the patient to feel comfortable discussing issues of violence while receiving medical care. A Brazilian group studied the consequences of domestic violence on woman's health during climacterium, administering a questionnaire to 124 patients who had suffered some form of violence during their lifetime. Of these, 80.6% did not reach out for medical care after an incident of violence. A total of 75% of the women interviewed indicated that if a medical professional had raised the matter of violence during the consultation, they would have asked for help. The study demonstrates that patients, if given a safe space to discuss issues of violence, would utilize the opportunity. It also highlighted that a majority of women believe professionals should encourage them to be more open about the matter and to proactively report any violence suffered.

It is also important to acknowledge that the violence suffered through life often impacts negatively on the victim's health, both physically and psychologically, lowering their quality of life and contributing to the genesis and/or aggravation of diseases. The same Brazilian study indicates a relation between the kind of aggression endured and the comorbidities in climacterium. The women who suffered physical violence had higher rates of depression and chronic immunological diseases. Those who suffered sexual violence more frequently presented depression and fibromyalgia; in addition, they also had a higher intensity of climacterium symptoms. Notably, 90.3% of the women interviewed had a negative

impact on their quality of life and behavior due to the violence suffered, and only 7.87% of them reported of having a satisfactory sexual life.

Concerning the limitations of the study, due to the descriptive and observational methodology used, we could not infer that the knowledge demonstrated by physicians is really applied in daily practice. Also, due to the type of questionnaire employed, physicians with a stronger interest in the subject matter may have been selected due to sampling bias. Another difficulty encountered was the evaluation of institutional resources, which were only inferred from the physicians' responses to the questions posed.

The main strength is to adopt the weaknesses in the health care of these patients mainly what concerns to lack of institutional support, raising a delicate but extremely important issue like violence against women, to engage health services to act as a source of not only treatment but also care, prevention, and reporting violence.

CONCLUSION

Most of the interviewed physicians recognize the importance and have sufficient knowledge to identify and treat victims of violence. However, the majority of them found it difficult to adequately deal with such cases due to the lack of preparedness of the multidisciplinary team, the restricted time frame of consults, and above all, the lack of institutional support.

As the emergency room is the first care, the professionals must be capable of recognizing the victims of violence and know how to refer them to a multiprofessional network.

AUTHORS' CONTRIBUTIONS

DDAM: Conceptualization, Data curation, Formal Analysis, Formal Analysis, Writing – review & editing. **GGFR:** Formal Analysis, Formal Analysis. **GSG:** Formal Analysis, Formal Analysis. **EJT:** Conceptualization, Formal Analysis, Formal Analysis, Writing – review & editing.

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ORIGINAL ARTICLE

AOP

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Shear wave elastography in early diabetic kidney disease

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SUMMARY

OBJECTIVE: This study aimed to analyze the kidneys among the subjects with early stages of type 2 diabetic kidney disease by shear wave elastography quantitatively.

METHODS: A total of 108 patients with type 2 diabetic kidney disease and 17 control subjects were enrolled. According to the estimated glomerular filtration rate and urinary albumin-to-urinary creatinine ratio, patients were classified into stages 1 to 3 diabetic kidney disease. Grayscale ultrasound andshear wave elastography were performed. The sizes, depths, and shear wave elastography values were recorded. These parameters were compared between the diabetic kidney disease and the control subjects.

RESULTS: The mean shear wave elastography values were significantly higher in the diabetic kidney disease group (10.156±1.75 kPa vs. 8.241±1.4 kPa; p<0.001). We obtained statistically significantly higher shear wave elastography values in stages 2 and 3 diabetic kidney disease subjects than control subjects and in patients with stage 3 diabetic kidney disease compared to those with stage 1 diabetic kidney disease (p<0.05 for all). We obtained a cutoff value of 9.23 kPa for predicting diabetic kidney disease in early stages, with a sensitivity of 67% and a specificity of 82%.

CONCLUSION: Shear wave elastography may be used as a noninvasive, simple, and quantitative method to provide diagnostic information as a part of routine management of patients with type 2 diabetes mellitus, especially in the early stages of diabetic kidney disease.

KEYWORDS: Diabetes mellitus. Kidney. Ultrasonography. Elastography.

INTRODUCTION

Diabetic kidney disease (DKD) is the leading cause of chronic kidney disease (CKD)[1]. It can be controlled or even be reversed if timely diagnosis and treatment are provided. Serum creatinine, albuminuria, and estimated glomerular filtration rate (eGFR) were less reliable indicators in early stages[2]. Biopsy provides a definitive diagnosis, although it has life-threatening complications. Computed tomography (CT) and magnetic resonance imaging (MRI) can evaluate morphological and functional situation of the kidney. However, they have some disadvantages, such as higher costs, long appointment time, radiation exposure, contrast-induced nephropathy, or nephrogenic systemic fibrosis. Ultrasound (US) is a noninvasive, available, cheap, and frequently used method to evaluate the kidney. US findings might be helpful especially in advanced stages such as decreased renal size, parenchymal thickness, and increased parenchymal echogenicity[3]. Early stages are reversible, but the most common diagnostic problems appear in these stages. Because in the hyperfiltration stage, the size of the kidney is normal, even bigger and the parenchymal thickness and the echogenicity are usual[4]. There is a requirement of a noninvasive method for the evaluation of DKD in the early stages.

Advanced, noninvasive, and simple sonographic techniques such as shear wave elastography (SWE) have been improved to identify the development of parenchymal fibrosis quantitatively based on the stiffness. In SWE, the transducer applies a transient acoustic radiation force to deform the tissues. Deformed waves, also known as shear waves, measured in meters per second and converted into a quantitative stiffness score in kPa by using Young's modulus, radiating in a perpendicular direction to the US beam. A low speed corresponds to soft, while a high speed indicates a stiff medium. To the best of our knowledge, there is little information about renal stiffness in the early stages of DKD. This study aimed to investigate the SWE technique for the quantitative assessment of DKD in early stages.

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METHODS

This prospective study was approved by the Research and Ethics Committee of our institution (approval number: 17-KAEK-100) and written informed consent was acquired from participants. The inclusion criteria of the study group were the patients with type 2 diabetes mellitus (DM) who had stages 1–3 DKD. The control group included age- and sex-matched healthy subjects who had no CKD, DM, hypertension, and cardiovascular disease. Subjects with (1) other primary renal diseases such as cyst, stone, and hydronephrosis; (2) malignancy; (3) pregnancy and lactation; (4) mental illness; (5) obesity with a renal depth more than 8 cm from the skin surface; (6) thin renal parenchymal thickness; (7) could not hold breath according to the radiologists' instructions; (8) solitary kidney; and (9) <30 eGFR levels were excluded. Between April and November 2018, we evaluated 108 consecutive subjects (36 males, 72 females; mean age±SD=56.3±10.6 years; age range: 20–85 years) who were admitted to the Department of Endocrinology. The control group comprised 17 subjects (10 males, 7 females; mean age±SD=56.8±9.3 years; age range: 43–73 years) and were recruited from the study site.

Clinical and laboratory data

We obtained demographic features and serum creatinine, blood urea nitrogen (BUN), and spot urinary albumin-to-urinary creatinine (UA/UC) ratio, within 1 week of undergoing the SWE. The diagnosis and the classification of DKD were established based on the "A new Classification of Diabetic Nephropathy 2014: a report from Joint Committee on Diabetic Nephropathy." This classification was based on the eGFR and UA/UC ratio[5]. We calculated eGRF for the serum creatinine concentration and age, using the new abbreviated equation of Modification of Diet in Renal Disease (aMDRD) for Turkish patients as follows: eGFR = aMDRD = 186 × (serum creatinine) – 1.154 × (age) – 0.203 × 0.742 (if female). According to the UA/UC, normoalbuminuria is defined as the levels <30 mg/g, microalbuminuria is defined as the levels between 30 and 300 mg/g, and macroalbuminuria is defined the levels >300 mg/g.

Sonographic evaluation

A radiologist with 15 years of experience who were blinded to the groups performed examinations. All subjects were fasted at least for 4 h and instructed to urinate before the examination. Ultrasonography and SWE examinations were performed in the right and left lateral decubitus positions during maximum inspiration to minimize kidney movement and obtain a full-size image of each kidney by using a Logic E9 system (GE Medical Systems, Milwaukee, WI, USA) with C1-6-D XDclear 1–6 MHz broadband convex transducer. Grayscale settings were adjusted to have optimum brightness, contrast, and increased spatial and temporal resolution. Length, width, and depth of each kidney were measured. Transducer placed longitudinally with minimal compression and SWE software turned on. On grayscale, an elastographic box with a size of 10 mm×10 mm was manually positioned, and stiffness results were coded in a color-coded map (Figure 1). Nine consecutive 5-s cine clips were conducted from the upper-lower pole, and the midportion of renal cortex, excluding vessels. At postprocessing period on the same equipment, a circle-shaped region of interest (ROI) was placed into the box above mentioned, and measurements were conducted in kPa. The mean SWE value of both kidneys was recorded. On average, sonographic evaluation period was about 20 min and the postprocessing period was about 10 min.

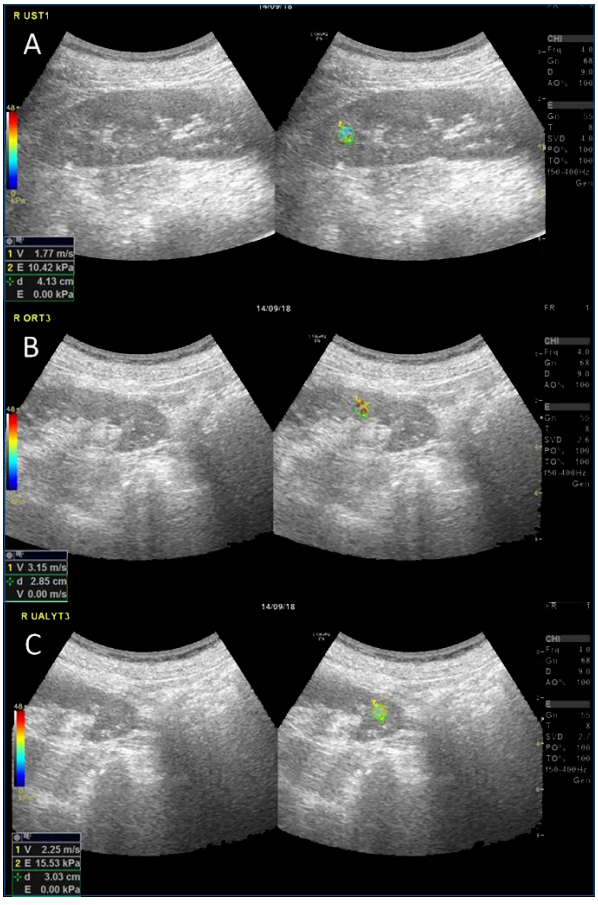

Figure 1. (A–C): Examples of shear wave elastography measurements performed from different parts of kidney on longitudinal scan in an a-73-year-old woman with type 2 diabetes mellitus and stage 3 diabetic kidney disease.

Statistical analyses

The normally distributed variables were shown as mean±standard deviation (SD), and non-normally distributed variables were stated as median [interquartile range (IQR)]. Categorical data were shown as numbers with related percentages (n, %) and compared by using the chi-square test. The differences in continuous variables were analyzed by using the Student's t-test or Mann-Whitney U test. The means of three or more samples were compared with one-way analysis of variance (ANOVA) test. The correlations between laboratory parameters, gray-scale, and SWE parameters were evaluated with the Pearson's and Spearman's bivariate correlation (r) tests. Receiver operating characteristic (ROC) curves were carried out, and the areas under the curve (AUCs) were estimated in order to investigate the role of SWE values for the distinction and staging of DKD. The sensitivity, specificity, and cutoff values from the closest point to the left upper corner on the ROC curve, with 95% confidence intervals (95%CIs) were obtained. A $p<0.05$ was considered statistically significant. All statistical analyses were performed by using SPSS (Statistical Package for the Social Sciences) statistical software package (version 11: SPSS Inc., Chicago, IL, USA).

RESULTS

There was no statistically significant difference in the age, gender, and renal length, width, depth between both groups ($p>0.05$ for all), but the renal SWE values were significantly higher in the patient group ($p<0.001$). ANOVA showed statistically significant increased renal SWE values in patients with stages 2 and 3 DKD compared with control subjects ($p<0.001$ for all) and in patients with stage 3 DKD than those with stage 1 DKD ($p=0.006$) (Table 1). Pearson's correlation coefficient revealed a weak positive correlation between albuminuria and the mean renal SWE values ($r=0.22$, $p=0.026$). Among the renal SWE values, the most sensitive cutoff value was 9.23 kPa between control subjects and patients with stage 3 DKD (sensitivity, 74%; specificity, 82%) (Table 2).

Table 1. Depth, length, width, and SWE values of kidneys in both groups.

	Type 2 diabetes mellitus	Control subjects	p
Depth (mm) (mean±SD)	38.7±9.48	43.2±7.33	0.064
Length (mm) (mean±SD)	103.88±12.33	101.97±9.93	0.546
Width (mm) (mean±SD)	47.44±6.74	47.92±5.72	0.781
SWE values of both kidneys (kPa) (mean±SD)	10.156±1.75	8.241±1.40	<0.001
Control subjects	8.241±1.404		
Stage 1 DKD[a]	8.948±0.799		
Stage 2 DKD[a]	10.239±1.784		
Stage 3 DKD[b]	10.572±1.804		

[a]p=0 compared with the healthy control group; [b]p=0.006 compared with DKD in stage 1. SWE: shear wave elastography; DKD: diabetic kidney disease; SD: standard deviation.

Table 2. Sensitivity, specificity, and cutoff values of SWE for predicting the presence and stage of diabetic kidney disease.

	Area under the curve	p	Cutoff value of SWE (kPa)	Sensitivity (%)	Specificity (%)
Mean value of both kidneys					
Stages 1 and 3 DKD	0.798	0	10.166	56	100
Control and stage 2 DKD	0.801	0	9.38	65	82
Control and stage 3 DKD	0.851	0	9.23	74	82
Control and DKD	0.798	0	9.23	67	82

SWE: shear wave elastography; DKD: diabetic kidney disease.

DISCUSSION

Albuminuria often indicates glomerular dysfunction, which is a characteristic of DKD in type 1 DM. However, tubulointerstitial fibrosis and vascular lesions are related to the development of DKD in type 2 DM. Some studies reported normal albuminuria levels in advanced CKD with type 2 DM[6,7]. Also, albuminuria and serum creatinine levels might be affected by diet, menstruation, muscle mass, and exercise. eGFR has a limited role in the hyperfiltration phase[8,9]. Due to the limitations of these laboratory methods in the early diagnosis of DKD, some imaging methods have been developed.

Grayscale US findings might be challenging in the early stages of DKD[3]. In early stages, due to hyperfiltration, US shows "bigger" and "better" kidney in patients with DKD compared to the kidney with the same level of chronic renal diseases[4]. It is reported that renal length, parenchymal thickness, and parenchymal echogenicity were not useful to indicate the severity of the DKD[10]. The measurement of size might be influenced by hydration. Evaluation of parenchymal echogenicity is a subjective and non-quantitative method. In recent years, new and quantitative sonographic methods such as elastography have been developed that could be helpful to demonstrate functional impairment[11-17].

We found significantly increased stiffness values in the patient group than healthy subjects (10.156±1.75 kPa vs. 8.241±1.40 kPa). Hassan et al. reported increased cortical stiffness in patients with advanced DKD compared to healthy subjects (23.72 kPa vs. 9.02 kPa) and in patients with stage 4 compared to those with stage 3 (30.4 kPa vs. 14.6 kPa)[15]. The reason for these higher values might be due to the fact that their study group consisted of patients with stages 3 and 4, in contrast to our study which included patients with stages 1–3. Lin et al. found increased parenchymal stiffness in the later stages of DKD[17]. Samir et al. obtained higher median SWE values (9.40 kPa) in patients with CKD who mostly comprised patients with DM[18]. To the best of our knowledge, there are a few studies about the diagnosis of DKD in the early stages[14,19]. Similar to our results, Liu et al. reported increased SWE values in the early (7.93 kPa) and middle stages (16.88 kPa) of patients with DKD compared to the diabetic subjects without DKD (5.51 kPa)[14]. Koc et al. reported increased stiffness in subjects with type 2 DM without diabetic nephropathy compared to healthy subjects (9.86 kPa vs. 7.92 kPa)[19]. Similar to our results, Goya et al. observed increased shear wave velocity values in DKD[16]. They obtained the highest shear wave velocity values in patients with stage 2, in contrast to our result on patients with stage 3. We found a progressive increase in the SWE values between stages 1 and 3 that might be attributed to the increase in fibrosis. However, they reported a progressive decrease in shear wave velocity values between stages 2 and 5 DKD. They stated this decrease might be related to renal function[16]. Since patients with stages 4 and 5 were not included in our study, we do not know the exact relationship of stages of DKD and SWE.

The validation of a cutoff SWE value in the investigation of early DKD might allow closer follow-up and planning of treatment. In our opinion, a cutoff value of 9.23 kPa might be considered in the diagnosis of early DKD.

Our results showed a weak positive correlation between SWE values and albuminuria. This is also accordant with previous research findings[15,16]. Some studies reported a relationship between cortical stiffness values and eGFR, BUN, and serum creatinine[12,15,16,19]. These discrepancies may conclude that eGFR is not an early marker of kidney damage, while BUN and serum creatinine levels are useful in the initial diagnosis of acute or chronic kidney disease and not monitoring of CKD[20,21].

This study has many limitations. One limitation of our study is that the analyses were performed by a single radiologist. Intra- and inter-observer agreement rates were not evaluated. The reason for this conflict is the long duration of the examinations. Another limitation is that SWE may be affected by movement artifacts, and a maximum depth of 8 cm limits the use of this method. Because of the fact that recruiting the age-matched subjects without any other chronic disease affecting kidneys and malignancies was challenging, the number of control subjects was limited. Another significant limitation is the lack of a gold standard, such as histopathological results. Performing the histopathological confirmations would increase the cost of the study and not be ethical and legal for the patients. Finally, the follow-up clinical, laboratory, and SWE results of the patients are not available.

CONCLUSIONS

Renal stiffness measured by SWE may be used as a noninvasive, simple, cost-effective, quantitative, and reliable imaging method to provide extra diagnostic information as a part of the routine sonographic investigation of patients with type 2 DM to reveal the early the changes in DKD. Despite its limitations, SWE imaging is a promising method that can be integrated with traditional laboratory methods in daily routine practice.

AUTHORS' CONTRIBUTIONS

RY: Conceptualization, Data curation, Investigation, Methodology, Writing – original draft. **FC:** Formal Analysis, Investigation, Writing – review & editing. **MY:** Formal Analysis, Methodology, Writing – review & editing. **FK:** Data curation, Resources, Writing – review & editing.

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ORIGINAL ARTICLE

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Profile of hemotherapy care and the safety of the transfusion process

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SUMMARY

OBJECTIVE: This study aimed to evaluate the safety of the transfusion process in a public teaching hospital and to outline the profile of the hemotherapy care provided.

METHODS: This was an exploratory, descriptive, and prospective study with a quantitative approach and grounded in field research. Data were obtained from medical and nursing records and active search.

RESULTS: Concentrated red blood cells were the most transfused blood component. Inadequate indications of blood components were detected in 15% of Concentrated red blood cells transfusions, 20% of fresh plasma, 29.2% of platelet concentrates, and 36.4% of cryoprecipitates. Filling out the blood component request forms, the nursing checklist and the entry book were inadequate in 88.3, 92.8, and 69.5% of the procedures, respectively.

CONCLUSIONS: Faults were identified throughout the transfusion process, revealing inadequate compliance with current standards and legislation, essential in minimizing the occurrence of errors and maximizing the safety of transfusion. Studies of this nature reinforce the need for continued research in this field.

KEYWORDS: Patient safety. Blood component transfusion. Hospitals. Blood safety.

INTRODUCTION

Blood transfusion is an effective therapeutic method, universally used, with a specific indication and guideline for each blood component (BC). However, even if all procedures for the safety of the transfusion process are adopted, risks remain. It is a high-cost practice for public health systems, due to the need for advanced technologies and specialized human resources. However, the rational use of blood is a universally defended approach in that it guarantees greater safety for both donor and recipient, is easily accessed by patients, and results, ultimately, in treatment cost reduction[1,2].

Given the risks inherent in the transfusion act, professionals need to be trained and aware of the importance of following protocols and current laws, enabling greater safety throughout the process. Disregarding one of the steps may lead to potentially fatal damage.

This study focused on analyzing the transfusion process in surgical patients at a university hospital, verifying the adequacy of the indication of BCs as well as the request of BCs, to assess the profile of assistance provided to these blood recipients.

The goal was to obtain support for the implementation of strategies that might contribute to the improvement of prescriptions, their careful use, and the administration of BCs, in order to increase transfusion safety.

METHODS

Type of study

This was an exploratory, descriptive, and prospective study with a quantitative approach, based on field research and grounded in field research.

Participants

Surgery patients from the HC/UFTM, who had received transfusions of BCs during hospitalization in the adult emergency department (PSA), orthopedics, surgical clinic, adult intensive care unit (AICU), coronary intensive care unit (CICU), surgical center, and postanesthesia care unit (PACU) participated in the research, from October 2018 to August 2019.

Inclusion criteria were as follows: surgical patients, ≥18 years old, transfused (conscious or unconscious), and those who received BCs and agreed (patient or family member) to participate in the study by signing the free informed consent form (FICF). Exclusion criteria were as follows: neurological and gynecological surgery patients and cases of death within the first 24 h.

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Data collection

In an active search 24 h after the transfusion, demographic and clinical–epidemiological data (e.g., sector, surgical specialty, type of BC, indication for transfusion, and quantity of BC, as well as information regarding the presence and filling out of forms and the book used in the registration of transfusion care) were noted on a spreadsheet designed for the study.

Data analysis/Statistics

A database was created using the Microsoft Office Excel 2010 application to implement the double-entry validation process. The database was imported into the SPSS (Statistical Package for the Social Sciences) program, version 2.1, which was used for a descriptive analysis by means of the analyses of absolute and relative frequencies, measures of central tendency (mean), and dispersion (standard deviation).

Ethical aspects

The study was approved by the Research Ethics Committee (REC) of the Hospital das Clínicas of the Federal University of Triângulo Mineiro (HC/UFTM), opinion no. 2.894.473.

RESULTS

A total of 393 transfusion procedures were evaluated in 184 patients who received 1042 BCs. Of these, 54.0% were male, 53.0% white, 38.0% brown, and 9.0% black; 19.5% were between the ages of 18 and 39 years, 26.5% were between 40 and 59 years, and 54.0% were 60 years or older. Hence, ages ranged from 18 to 94 years, and the average age was 57.74 (SD=19.21).

The most transfused BC was packed red blood cells (PRBCs) at (61.6%), followed by 16.0% of platelet concentrates (PCs), 14.0% of fresh plasma (FP), and 8.4% of cryoprecipitate (Cryo). Regarding the blood type, group O represented 44.3%, followed by A (40.0%), B (13.0%), and AB (2.7%), of which 90.6% were Rh positive. Regarding the indications for transfused BCs, based on the Protocol for the Rational Use of Blood of the HC/UFTM/2017, the inadequacy percentages for the PRBCs, PC, FP, and Cryo were 15.0%, 20.0%, 29.1%, and 36.4%, respectively. Furthermore, 4.2% of the FP, 15.0% of the PC, and 36.4% of the Cryo had their assessment impaired due to a lack of previous examination (Table 1).

Regarding the completion of the BC request forms, of the 377 evaluated, 4.1% were not located, 88.3% had some level of inadequacy, and 86.7% of the fields referring to clinical and epidemiological data had a higher level of noncompliance.

Regarding the nursing checklist, 387 transfusion events were evaluated (1.5% not found), and of these, 359 (92.8%) had some inadequacy. The main noncompliances were related to monitoring at 10 and 30 min after transfusion, with no record in 67.4% and 77%, respectively (Table 2).

The analysis of the records in the BC entry book, an instrument used to facilitate traceability, was carried out in 76.8% of the transfusion acts; however, 23.2% did not locate the sector. Of the 302 evaluated, completion inadequacy was 69.5%,

Table 2. Assessment of the adequacy of filling out the blood transfusion checklist by the nursing staff of the 359 transfusion acts analyzed. Uberaba (MG), 2019.

Aspects analyzed	Adequate n (%)	Inadequate n (%)
Patient's identification data	222 (61.8)	137 (38.2)
Sample identification data	276 (76.9)	83 (23.1)
VS–beginning of transfusion	283 (78.8)	76 (21.2)
VS–end of transfusion	243 (67.7)	116 (32.3)
Information about transfusion reaction	217 (60.4)	142 (39.6)
Checklist: VS evolution 10 min	98 (27.3)	261 (72.7)
Checklist: VS evolution 30 min	61 (17.0)	298 (83.0)

VS: vital signs.

Table 1. Inadequacy in the indication of blood components in the 393 transfusion procedures evaluated, Uberaba, MG, 2019.

Type of blood component	Transfusion act			
	Adequate n (%)	Inadequate n (%)	No assessment* n (%)	Total n (%)
Packed red blood cells	320 (85.0)	55 (15.0)	0 (0)	375 (100)
Fresh plasma	26 (65.0)	8 (20.0)	6 (15.0)	40 (100)
Platelet concentrate	16 (66.7)	7 (29.1)	1 (4.2)	24 (100)
Cryoprecipitate	3 (27.2)	4 (36.4)	4 (36.4)	11 (100)

*No assessment: Unable to assess.

with the main area of noncompliance relating to the transfusion reaction field, of which 66.9% were not filled in.

The term of acknowledgment and authorization of the transfusion was not found in almost all of the evaluated medical records (94.9%). As for the medical prescriptions, we observed that in most of these, dripping was not specified, and checking and double checking by the nursing staff was identified in, respectively, 84.4% and 40.9% of the 393 transfusion procedures evaluated. In 20.1% of these, BC was not prescribed (only the request for BCs was identified), and the vast majority of these nonprescriptions were provided during the surgical procedure.

DISCUSSION

Of the patients evaluated, most were male and aged over 60 years, coinciding with findings in the national literature[3,4]. However, a multicenter study carried out recently in Turkey recorded a higher percentage of women[5]. Blood type O was the most transfused, which is in line with findings in the literature[4] and with the profile of the Brazilian population. Data from the Seventh Hemotherapy Production Bulletin (Hemoprod 2018) indicated that 47.86% of donations made in the country that year were from the O group[6].

Regarding the distribution of transfusions by specialty, the BCs were the most frequently used in orthopedic surgery, followed by general surgery. As for the sectors, they were most used in the AICU and CICU, which is in agreement with the literature[4,7]. The most transfused BC was the PRBC, coinciding with national and international data[3,4,8,9].

A high index of inadequacy of indications for BCs was observed, especially for cryoprecipitate and PC, as is the case with other hospitals in the country[10,11]. However, a study[5] points out even higher rates of inadequacy in the indications for PRBC, corresponding to 99% preoperatively, 23% intraoperatively, and 43% postoperatively.

In a previous study conducted between 2007 and 2008 across 226 blood transfusion services located in 178 municipalities in Minas Gerais (Brazil), the presence and performance of the Transfusion Committees (TCs) was evaluated. These committees were implemented in 63.4% of the services visited. Nonconformities were recorded in the requests for BCs, identification of blood samples, and records of transfusions and observed to be significantly higher in services that did not have these committees. However, it was also found that the performance of these committees was still in the developmental phase[12].

These results demonstrate low compliance with protocols for the use of BCs, low performance of the TC and of the blood therapy service. TC became mandatory in the country in 2004[13]; however, its results are still limited[14].

The aforementioned legislation emphasizes the need to implement permanent training for medical and nursing staff, to increase the efficiency of the TC by implementing mechanisms for verifying and evaluating HC requests in the service and complying with the legislation. Such measures are essential to reverse the situation found in this study and to provide the rational use of this important therapeutic resource and increase transfusion safety.

As for the availability and use of the forms, it was observed in the units that they were frequently absent. Furthermore, there were numerous examples of noncompliance in their completion. The biggest instance of noncompliance found in the BC application form was in relation to clinical and epidemiological data and in the field referring to clinical indication and diagnosis. Very often, only the diagnosis was registered. Similar results were observed in a study carried out in 2015 in Tunisia[15].

Regarding the blood transfusion checklist, the very high percentage of nonconformities was noteworthy, especially in the item "Verification of Vital Signs at 30 min of transfusion," followed by "Record of Observation in the initial 10 min." Monitoring within the first 10 min of transfusion is essential for early detection of changes in vital signs in relation to pre-transfusion data and may be an opportunity to detect a possible serious reaction, such as hemolytic ABO incompatibility. Literature data corroborate our findings[4,16], demonstrating that the inadequate completion of the checklist is also a constant in different services, which can be attributed to the lack of awareness about the relevance of these records and also to a deficiency in monitoring these actions, reinforcing the importance of active TCs.

The entry book was the document revealing the greatest completion, which may be explained as due to its greater simplicity and lack of information. In particular, the registration of the BC bag number, which is of great importance for enabling its tracking, obtained a good adequacy index, demonstrating greater awareness by professionals of its importance, a factor not observed by Santos et al, at another large general hospital in 2013[16].

The Letter of Science and Authorization was not implemented by the vast majority of professionals. Similar results were observed in a reference hospital in England, in which a consent form was obtained for only 9% of transfusions[17]. Despite being a mandatory procedure in medical practice, as provided for in Article 6 of the Universal Declaration on Bioethics and Human Rights of UNESCO in 2005[18] and also in the codes of medical ethics of most countries, such as Brazil

in Article 22[19], it is noted that less importance has been given to this requirement.

The lack of BC prescription in the medical records was a frequent finding in transfusions performed intraoperatively. Similarly, the prescription of dripping was not identified in most procedures. Both inadequacies were also identified in the literature[16], demonstrating that such procedures, which are important for transfusion safety, are also not properly observed by teams involved in surgical procedures.

Another recurrent inadequacy was related to the checking of the bag and prescription data by the nursing staff. Despite the need for double-checking, this was observed in less than half of the procedures, and a single check was registered in just over 80% of cases. Similar results were observed in another study[16]. Such procedure, essential in confirming the execution of medical prescriptions, is another barrier in the prevention of adverse events by minimizing the chance of errors[20].

Our findings reinforce the need for a more careful assessment regarding the indication of BC in surgical patients, with a focus on restrictive hemotherapy and on patient blood management (PBM). The indications for PRBC deserve special attention, since iron deficiency anemia, the most common cause of chronic anemia, present in more than 50% of these patients, is a condition that can often be resolved before the surgical procedure[21]. Failures in the indication and prescription of BCs have been frequent, reflecting a gap in the knowledge of transfusion therapy. One study argues that *incremental levels of training in transfusion medicine during graduation may narrow the gaps between specialist and blood prescribers and possibly even encourage transfusion research*[22]. Furthermore, the high levels of inadequacy detected in confirming the execution of prescriptions, and in the nursing records, denote deficient training and/or noncompliance with legislation governing transfusion**.** Although the sample size was limited, the 184 patients and almost 400 transfusion events evaluated were considered sufficient for an adequate analysis of the hemotherapy practiced in the service and that the nonconformities, which compromise transfusion safety, are not just a local problem.

Thus, it is necessary that TCs start acting in the same way as the hospital infection committees (established in the country in 1997) by monitoring the hemotherapy practice. This can be done through an active search, with verification and evaluation of the transfusion requests and effects of adverse events, and strict compliance with the protocols and legislation related to hemotherapy, as well as the continuing education of the staff. Such measures are essential to reverse the situation found, promote the standard use of this important therapeutic resource, and increase transfusion safety.

CONCLUSIONS

We found that the risk management of the transfusion process in surgical patients at the HC/UFTM, as well as the attention to BCs, are not being performed properly, compromising patient safety. The results presented in this research point to the need for educational activities, by means of training at all stages of the transfusion process, directed at the entire team involved in this activity (doctors, nurses, and biomedical doctors), under the responsibility of the institution TC and the hemotherapy service. Studies of this nature reinforce the need for continued research in this field.

AUTHORS' CONTRIBUTION

JG: Conceptualization, Data curation, Formal Analysis, Writing – original draft, Writing – review & editing. **SSS:** Conceptualization, Formal Analysis, Writing – review & editing. **HMZ:** Conceptualization, Formal Analysis, Writing – review & editing. **JM:** Data curation, Formal Analysis, Writing – review & editing**.**

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ORIGINAL ARTICLE

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Comparison of perioperative indicators, treatment efficacy, and postoperative complications between tonsillotomy and tonsillectomy for children with obstructive sleep apnea hypopnea syndrome

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SUMMARY

OBJECTIVE: This study aimed to compare the perioperative indicators, treatment efficacy, and postoperative complications between tonsillotomy and tonsillectomy for children with obstructive sleep apnea hypopnea syndrome.

METHODS: A total of 134 children with obstructive sleep apnea hypopnea syndrome were divided into tonsillotomy group (n=66) and tonsillectomy group (n=68). The tonsillotomy group received tonsillotomy treatment with a power cutter, while the tonsillectomy group received tonsillectomy treatment. The perioperative indicators, treatment efficacy, and postoperative complications were compared between the two groups.

RESULTS: There was no significant difference in operative time between the two groups ($p>0.05$), with significant difference in amount of blood loss, postoperative Visual Analogue Scale score, food intake amount, and general diet-taking starting time between the two groups ($p<0.05$). The total effective rate of treatment had no significant difference between the two groups ($p>0.05$). There was significant difference in postoperative bleeding, upper respiratory tract infection, and pharyngeal scar grade between the two groups ($p<0.05$).

CONCLUSIONS: Compared with tonsillectomy treatment for children with obstructive sleep apnea hypopnea syndrome, tonsillotomy treatment is more beneficial to optimize the perioperative indicators, relieve the postoperative pain, facilitate the postoperative recovery, and reduce the postoperative complications, which is worthy of clinical promotion.

KEYWORDS: Tonsillectomy. OSAHS. Children.

INTRODUCTION

Obstructive sleep apnea hypopnea syndrome (OSAHS) is the most common disease that affects the sleep, snoring, and mouth breathing of people. It can cause the long-term hypoxia and facial malformations, thereby severely influencing the growth and development of children. The OSAHS in children is often related to the adenoidectomy and tonsil hypertrophy due to the airway obstruction[1]. According to the 2012 Guidelines of the American Academy of Pediatrics (AAP), the incidence of OSAHS in children is about 1.2–5.7%[2]. At present, the low-temperature plasma radiofrequency tonsillectomy (TE) is mostly used for the clinical treatment of children with OSAHS, which achieves the therapeutic purpose through complete removal of tonsils and/or adenoids but causes great trauma to the body and many complications such as postoperative pain and bleeding[3,4]. However, the tonsillotomy (TT) achieves the therapeutic purpose by removing part of tonsils with cold instruments, which can not only relieves the disease of children but also retains the basic function of tonsils and reduces the occurrence of postoperative complications[5-7]. In this study, the effectiveness of TT and TE in children with OSAHS was investigated, and the perioperative indicators, treatment efficacy, and postoperative complications were observed.

METHODS

Patients

A total of 139 OSAHS children with surgical indications admitted in our hospital from January 2020 to January 2021 were randomly divided into TT group (68 cases) and TE group (71 cases), which received dynamic TT and low-temperature plasma radiofrequency TE, respectively. The follow-up was performed for 9 months, and five cases were lost to follow-up. Finally, 134 cases were included in the study. In TT group, there were 40 males and 26 females and the age was 5.57±0.82 years. There

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were 48 cases with tonsil grade II and 18 cases with tonsil grade III. In TE group, there were 43 males and 25 females and the age was 5.46±0.83 years. There were 51 cases with tonsil grade II and 17 cases with tonsil grade III. There was no significant difference in clinical baseline data between the two groups (p>0.05). This study was approved by the Ethics Committee of Zhongshan Hospital affiliated to Xiamen University. Written informed consent was obtained from the families of children.

Inclusion criteria and exclusion criteria

Inclusion criteria were as follows:

(1) OSAHS was diagnosed with combined symptoms, signs, and polysomnography indicators as recommended in the 2020 Guidelines for the Diagnosis and Treatment of Obstructive sleep apnea in Children in China[8];
(2) the preoperative examination showed no contraindications of heart, liver, or kidney function; and
(3) the course of disease was more than 6 months.

Exclusion criteria were as follows:

(1) the patients had a history of acute tonsil infection within 3 weeks before surgery;
(2) the patients had coagulation dysfunction; and
(3) the patients had anemia or leukocyte abnormalities before surgery.

Surgical methods

All children were intubated with airway tube, with pillow under shoulder and head back. Davis mouth opener was placed and fixed. The oropharynx was exposed, and the tonsils were fully exposed. In TT group, Medtronic XPS3000 power system with 0° cutting head was used for TT layer by layer from the upper pole to lower pole of tonsils. The upper fossa was exposed and opened from inside to outside. About 10% of tonsil tissues were retained. The bipolar electrocoagulation was used to stop the blood flow. In TE group, the low-temperature plasma radiofrequency ablation system with MC40 frequency knife was used for TE. The cutting energy chose step 7, with step 5 for stopping bleeding. The tonsils were slowly cut along the tongue palatal arch mucosa step by step. The gap between tonsils and surrounding tissues was exposed. The tonsils were removed from top to bottom along the gap. The tonsil fossa was examined for checking the residual tonsil tissues and bleeding.

Observation of perioperative indicators

The operation time (min) and amount of blood loss were recorded. On the perioperative days 1, 3, and 7, the pain of patients was assessed using Visual Analogue Scale (VAS), with 0 point for no pain and 10 points for extreme pain. The food (cold liquid diet or semi-liquid diet) intake amount (ml) on the postoperative days 1 and 2 was counted. In addition, the general diet-taking starting time (days) was recorded.

Evaluation of treatment efficacy

The treatment efficacy was evaluated according to the 2007 Draft Guidelines for the Diagnosis and Treatment of Children OSAHS (Urumqi)[9]. The scoring was performed using apnea–hypopnea index (AHI) and lowest blood oxygen saturation ($LSaO_2$) as follows:

(1) cured: AHI was less than five times per hour; $LSaO_2$ was more than 0.92; the clinical symptoms almost disappeared;
(2) remarkably effective: AHI decreased by >50%; the clinical symptoms were significantly mitigated;
(3) effective: AHI decreased by 25–50%; the clinical symptoms were mitigated; and
(4) ineffective: AHI decreased by <25%; the clinical symptoms were not mitigated, and even aggravated.

The total effective rate was calculated as follows: total effective rate (%) = [(number of cured cases + number of markedly effective cases + number of effective cases) / total case number] × 100.

Observation of postoperative complications

The postoperative bleeding of patients was observed. The upper respiratory tract infection of patients within postoperative 9 months was recorded. In addition, the postoperative pharyngeal scar formation was observed, and its degree was graded as follows: grade I: the palatopharyngeal arch and palatopharyngeal arch were clear, without scar formation; grade II: the palatopharyngeal arch and palatoglossus arch were fused, with a small part of fusion and visible scars; grade III: the palatopharyngeal arch and palatoglossus were mostly fused and basically disappeared; and grade IV: there was obvious scar, with protruding mucosal surface.

Statistical analysis

Statistical analysis was performed using SPSS version 20.0 software. The measurement data were expressed as mean±standard deviation and were compared by independent sample t-test. The counting data were expressed by number and rate and were compared by χ^2 test. A p-value <0.05 was considered statistically significant.

RESULTS

Comparison of perioperative indicators between the two groups

There was no significant difference in operative time between the two groups (p>0.05), with significant difference in amount of blood loss, postoperative VAS score (day 1, day 3, day 7), food intake amount (day 1, day 2), and general diet-taking starting time between the two groups (p<0.05) (Table 1).

Comparison of treatment efficacy between the two groups

At the end of treatment, there were 27 cured, 25 remarkably effective, 10 effective, and 4 ineffective cases in TT group, with 28 cured, 27 remarkably effective, 11 effective, and 2 ineffective cases in TE group. The total effective rate in TT and TE groups was 93.94 and 97.06%, respectively, with no significant difference between the two groups (p>0.05) (Table 2).

Comparison of postoperative complications between the two groups

In TT group, there were 0 cases of postoperative bleeding, 5 cases of upper respiratory tract infection, 63 cases of pharyngeal scar formation with grade I, 3 cases of pharyngeal scar formation with grade II, and 0 cases of pharyngeal scar formation with grade III. In TE group, there were 6 cases of postoperative bleeding, 14 cases of upper respiratory tract infection, 39 cases of pharyngeal scar formation with grade I, 26 cases of pharyngeal scar formation with grade II, and 3 cases of pharyngeal scar formation with grade III. There was significant difference in postoperative bleeding, upper respiratory tract infection, and pharyngeal scar grade between the two groups (p<0.05) (Table 3).

DISCUSSION

As one of the most common diseases in children, the OSAHS has attracted wide attention. This study investigated the effectiveness of TT and TE in children with OSAHS. Results showed that, compared to TE group, TT group had significant advantages in postoperative VAS score, food intake amount, and general diet-taking starting time. TE is performed at the tonsillar capsule. Elinder et al.[10]. have reported that, in TE, the thermal damage depth of tissue by plasma radiofrequency knife reaches 0.7–0.8 mm. In the process of separation, cutting, or coagulation at the capsule level, it is inevitable that part of the capsule will be damaged and the deep soft tissue

Table 1. Comparison of perioperative indicators between two groups.

Group		TT	TE	t	p
n		66	68		
Operation time (min)		16.77±2.14	16.09±2.01	1.897	0.060
Amount of blood loss (mL)		23.20±4.85	18.07±6.11	5.373	<0.001
VAS score (points)	Day 1	4.81±1.30	5.65±1.32	3.710	<0.001
	Day 3	4.92±1.12	6.27±0.93	7.600	<0.001
	Day 7	2.44±1.04	3.24±0.88	4.812	<0.001
Food intake amount (mL)	Day 1	830.31±124.07	669.13±88.17	8.689	<0.001
	Day 2	1115.92±159.66	801.50±137.12	12.241	<0.001
General diet-taking starting time (days)		6.90±0.82	12.70±1.73	24.677	<0.001

TT: tonsillotomy; TE: tonsillectomy; VAS: visual analogue scale.

Table 2. Comparison of treatment efficacy between two groups.

Group	n	Cured n (%)	Remarkably effective n (%)	Effective n (%)	Ineffective n (%)	Total effective rate (%)
TT	66	27 (40.91)	25 (37.88)	10 (15.15)	4 (6.06)	93.94
TE	68	28 (41.18)	27 (39.71)	11 (16.18)	2 (2.94)	97.06
χ^2						0.762
p						0.383

Table 3. Comparison of postoperative complications between two groups, n (%).

Group		TT	TE	χ^2	p
n		66	68		
Postoperative bleeding	Yes	0 (0.00)	6 (8.82)	6.097	0.014
	No	66 (100.00)	62 (91.18)		
Upper tract respiratory infection	Yes	5 (7.58)	14 (20.59)	4.660	0.031
	No	61 (92.42)	54 (79.41)		
Pharyngeal scar grade	I	63 (95.45)	39 (57.35)	26.748	<0.001
	II/III	3 (4.55)	29 (42.65)		

TT: tonsillotomy; TE: tonsillectomy.

and muscle tissue will be damaged. Deglutition, stimulation exposed to oropharyngeal muscles, nerve broken end, causes severe pain. TT adopts the dynamic cutter equipment for cold, which reduces the thermal damage and retains the tonsil capsule. The cutting edge is only limited at the peripheral sensory nerve. After TT, the tonsil tissue oropharyngeal secretions can be retained so that the postoperative pain is light and the time returning to normal eating is short[11,12]. However, the operation time of TT was slightly prolonged, which is considered to be related to surgical proficiency and wound size. The amount of blood loss in TT was slightly higher than TE. It is considered that the wounds of TT are on the surface of glands, resulting in more blood oozing. However, TE is mainly operated along the capsule. If the surgical skill is good, the main blood vessels will be blocked during the operation to stop bleeding and therefore the amount of blood loss is reduced.

In our study, both TT and TE showed significant efficacy in relieving the OSAHS, with no significant difference in total effective rate between the two groups. These results are consistent with previous studies[13,14]. Eviatar et al.[15] had made a long-term comparison between these two surgical procedures and confirmed that there was no significant statistical difference between TT and TE in many aspects including snoring, night suffrage, recurrent tonsillitis, hyperplasia of tonsils, or postoperative sleep monitoring indicators. Blackshaw et al.[16] showed that TT with capsule preservation was effective in the treatment of OSAHS in children.

Results of our study showed that the postoperative complications in TT group were significantly lower than TE group. The tonsil blood supply comes from five branches of the external carotid artery, but only the tonsil branch of facial artery enters the parenchyma from the lower pole of tonsil, so the bleeding during and after TE mostly comes from the lower pole of tonsil[13]. TT preserves the tonsil capsule. During the operation, only the facial artery branch entering the tonsil tissue needs to be completely hemostatic, without damaging the other branches. TE is operated under the capsule, which requires hemostasis of all branch vessels. Although the plasma system is operated at low temperature, the tissue thermal damage can still be as deep as 0.7–0.8 mm, resulting in partial collagen degeneration and forming a thin pseudomembrane covering the wound, temporarily protecting the wound from bleeding. In terms of postoperative infection, the incidence of upper respiratory tract infection in TT group was lower than that in the TE group, which may be caused by the retention of tonsil tissue and immune function of tonsil in TT group[16].

CONCLUSION

Compared with TE treatment for children with OSAHS, TT treatment is more beneficial to optimize the perioperative indicators, relieve the postoperative pain, facilitate the postoperative recovery, and reduce the postoperative complications, which is worthy of clinical promotion. This study still has some limitations. The sample size is relatively small, which may affect the persuasiveness of the results. In future studies, the sample size should be further increased for obtaining more satisfactory outcomes.

AUTHORS' CONTRIBUTIONS

HY: Conceptualization. **CJ:** Data curation, Formal Analysis. **XW:** Writing – original draft. **YH:** Writing – review & editing

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ORIGINAL ARTICLE

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Predictive factors for success after supine percutaneous nephrolithotomy: an analysis of 961 patients

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SUMMARY

OBJECTIVE: The aim of this study was to evaluate the predictive factors for success following percutaneous nephrolithotomy in the supine position.

METHODS: Patients who underwent percutaneous nephrolithotomy in the supine position from June 2011 to October 2018 were evaluated. Age, sex, body mass index, the American Society of Anesthesiologists physical status classification, hemoglobin level, number of previous surgeries, stone size, and the Guy's Stone Score were analyzed. Success was considered if no fragments were observed on the computed tomography scan on the first postoperative day. Univariate and multivariate analyses were performed to determine significant parameters.

RESULTS: We evaluated 961 patients; of them, 483 (50.2%) underwent previous stone-related surgery, and 499 (51.9%) had Guy's Stone Score 3 or 4. The overall success rate in a single procedure was 40.7%, and complication rate was 13.7%. The univariate analysis showed that the maximum diameter of the stone (25.10±10 mm; p<0.001), previous percutaneous nephrolithotomy (OR 0.52; p<0.001), number of previous percutaneous nephrolithotomy (OR 0.15; p<0.001), the Guy's Stone Score (OR 0.28; p<0.001), and the number of tracts (OR 0.32; p<0.001) were significant. In the multivariate analysis, the number of previous percutaneous nephrolithotomy (OR 0.54; p<0.001) and the Guy's Stone Score (OR 0.25; p<0.001) were statically significant.

CONCLUSIONS: Guy's Stone Score and the number of previous percutaneous nephrolithotomy are predictors of success with the supine position. Complex cases and with previous percutaneous interventions may require technical improvements to achieve higher stone-free rates.

KEYWORDS: Computed tomography. Kidney stone. Nephrolithiasis. Percutaneous nephrolithotomy. Supine position.

INTRODUCTION

The complete removal of kidney stones is the main objective in treating urinary stones. Failure can lead to complications, increased readmission rates, reoperation, and economic implications for the patients and the health system[1]. To date, stone size is the major parameter for choosing the treatment method, and percutaneous nephrolithotomy (PCNL) is currently recommended for kidney stones of up to 20 mm by the European Association of Urology and American Urological Association guidelines[2].

First described in 1976 by Fernström and Johansson[3], the PCNL in prone position was followed by the first supine position technique description in 1987[4]. The technique evolved, new equipments and endoscopes allowed better outcomes, and decreased complication rates. Comparing positioning, both have similar success rates, although recently, the supine approach has become more widely accepted. The possibility of performing all procedures in the supine position, its easy anesthetic management, and a safe profile are positive characteristics[5-7].

Several parameters may affect the stone-free rate such as the stone size, density and complexity, the anatomical variations, and the patient profile (e.g., body mass index [BMI] and comorbidities). Recent reports suggest that greater sensitivity and specificity make computed tomography (CT) the best tool to evaluate success[8-11].

To address this knowledge, we conducted a study to define predictors of stone-free rate after PCNL in the supine position in a large series of patients, evaluated by CT scan.

METHODS

A retrospective analysis of prospectively collected data was performed including all consecutive adult patients who underwent supine PCNL between June 2011 and October 2019 in a single center. Informed consent was obtained from patients preoperatively, and the study protocol was approved by the local ethics committee (institutional review board number: 8258117.8.0000.0091).

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Indications for surgery were single or multiple renal stones >2 cm in size and symptomatic stones <2 cm wherein first-line techniques (shockwave lithotripsy or ureteroscopy) failed. Prior to surgery, the variables analyzed were age, sex, BMI, American Society of Anesthesiologists (ASA) physical status classification, hemoglobin level, previous surgeries, stone diameter (maximum diameter defined as the cumulative size of the stones), history of spina bifida or spinal injury, and the Guy's Stone Score (GSS). The GSS, routinely evaluated in all cases, was determined by a urologist during the preoperative consultation by CT scan analysis and was confirmed just before the surgery. All urologists were previously trained in GSS.

Operative technique

All the supine PCNL procedures were performed under general anesthesia. Beginning with cystoscopy and placement of a 6-Fr ureteral catheter, a retrograde pyelogram and subsequent calyceal puncture were performed by the main surgeon under fluoroscopic and ultrasonic guidance. Subcostal skin punctures were preferred, although supracostal punctures through the 11th and 10th intercostal spaces were also used when necessary. Semirigid plastic dilators set (Amplatz dilators®) were used to sequentially dilate the tract up to 30 Fr. Nephroscopy was performed with a 26-Fr nephroscope (Karl Storz®, Germany), and stone fragmentation was performed with an ultrasonic lithotripter (Swiss Lithoclast Master®, EMS, Switzerland).

Intraoperative stone-free status was verified with fluoroscopy and flexible nephroscopy. A 16-Fr nephrostomy tube was placed at the end of the procedure in cases of bleeding, residual stones, solitary kidney, pelvic injury, or multiple tracts. Routinely, a 6-Fr ureteral catheter and 18-Fr bladder catheter were left in place until the first postoperative day (POD1); in cases of ureteropelvic junction edema or injury, a 4.8-Fr × 26-cm ureteral stent was used for 3 weeks. Of note, 20 mL of 1% ropivacaine was injected on the tracts at the end of the surgery.

Outcome evaluation

A low-dose non-contrast CT scan was routinely performed on POD1 in all cases. The success rate was defined as the absence of any residual fragments (RFs) (i.e., stone-free rate).

Statistical analysis

Software R Core 3.5.1 (Microsoft®, USA) was used for statistical analysis. Continuous variables were described by mean and standard deviations. Categorical variables were described by simple and relative frequencies. Odds ratio (OR) were presented using logistic regression. For the variables with a lower number of observations, the Fisher's test was used. Statistical significance was set at 0.05.

RESULTS

We enrolled 961 patients in the study. The mean age and BMI were 48.8±12.6 years and 27.4±5.1 kg/m², respectively (Table 1); 483 (50.2%) patients had previous stone-related surgery, and 499 (51.9%) had GSS 3 or 4 (complex cases). The overall success rate in a single procedure was 40.7% (Table 2), and the complication rate was 13.7%.

A univariate analysis of the continuous variables targeting the success outcome observed a statistical significance in maximum diameter (OR 0.95 [0.94 – 0.96]; p<0.001). The median size for RFs was 15.2±9.3 mm. There was no statistical significance in BMI (Table 2).

In the univariate analysis, previous PCNL (OR 0.52 [0.36; 0.75]; p<0.001), the number of previous PCNL (OR 0.15 [0.13; 0.33]; p<0.001), the GSS (OR 0.28 [0.18; 0.42]; p<0.001), and the number of tracts (OR 0.32 [0.21; 0.46]; p<0.001) were significant (Table 2).

After choosing the variables with statistical significance and performing a multivariate analysis, ORs and p-values were obtained, and the number of previous PCNL (OR 0.54 [0.42; 0.69]; p<0.001) and the GSS (OR 0.25 [0.13; 0.47]; p<0.001) were found to be significant (Table 3).

Table 1. Patient demographic and clinical characteristics.

	Total (n=961)
Sex; n (%), Female	551 (57.3)
Age; Mean (SD)	48.8 ± 12.6
BMI (kg/m²); Mean (SD)	27.4 5.1
Mean stone size; Mean (SD)	28.8±11.9
ASA; n (%)	
1	319 (33.2)
2	553 (57.5)
3 or more	89 (9.3)
Interventional stone treatments; n (%)	
None	468 (48.7)
Open surgery	44 (4.6)
ESWL	154 (16.1)
PCNL	184 (19.1)
Others	111 (11.5)
Guy's Stone Score; n (%)	
1	192 (19.9)
2	270 (28.1)
3	335 (34.9)
4	164 (17.1)

BMI: body mass index; ASA: American Society of Anesthesiologists; ESWL: external shock wave lithotripsy; PCNL: percutaneous nephrolithotomy.

DISCUSSION

Several factors influenced the previous underuse of supine PCNL, among them, the lack of experience in most urology centers[12] and the fear of colonic injuries. However, this scenario has been changing worldwide, and approximately 20% of centers use this technique currently[13], reaching up to 45% in certain locations[14] and 38.9% in Latin America[15]. Any of the supine position variations do not have an impact on success or complications compared to the prone position[16], and the supine position can be easily learned when training is done in a proper center.

This study involves 961 patients, operated in a single center, by 6 surgeons. All the surgeons have experience in both prone and supine positions and in using the standard technique in all cases. In the univariate analysis, the stone diameter, the time of fluoroscopy, the operative time, and the drop of hemoglobin were associated with residual stones. However, some of these factors (i.e., fluoroscopy and operative time) cannot be determined as a cause as they are essentially a consequence of more complex cases, which are reportedly associated with lower success rates[17]. The stone diameter was proven to be a predictor of success as shown in a study by Pérez-Fentes et al.[18] when stone burden was described as a predictor of being stone free. BMI has been demonstrated to not influence success in supine PCNL[19].

Previous kidney surgery, previous PCNL, and the number of previous PCNL had a negative impact on success rates, probably due to anatomic variations in the urinary tract such

Table 3. Multivariate analysis for predictive factors for success.

	Odds ratio [95%CI]	p-value
Number of previous PCNL	0.545 [0.423–0.689]	<0.001
Guy's Stone Score 2	0.337 [0.220–0.512]	<0.001
Guy's Stone Score 3	0.211 [0.134–0.328]	<0.001
Guy's Stone Score 4	0.250 [0.129–0.475]	<0.001
Number of tracts	0.975 [0.729–1.285]	0.861
Mean stone size	0.986 [0.972–1.001]	0.069

AUC 0.766 [0.736; 0.796]; PCNL: Percutaneous nephrolithotripsy.

Table 2. Univariate analysis according to outcoming stone free.

Variables	Stone-free status		Odds ratio [95%CI]	p-value
	Any residual stone (n=570)	Stone free (n=391)		
Age (years)	48.2±12.5	49.8±12.7	1.01 [1.00–1.02]	0.046
BMI (kg/m²)	27.3±5.3	27.4±4.9	1.01 [0.98–1.03]	0.85
Drop in hemoglobin level (g/dl)	2.2±1.4	1.9±1.2	0.82 [0.74–0.91]	<0.001
Mean stone size (mm)	31.4±13.7	25.2±9.3	0.95 [0.94–0.96]	<0.001
Hospital stay (h)	63.1±41.7	52.4±50.8	0.99 [0.99–1.00]	0.03
Operative time (min)	117.7±47.5	91.1±45	0.98 [0.98–0.99]	<0.001
Fluoroscopy time (min)	11.7±4.8	10.5±3.8	0.94 [0.92–0.95]	<0.001
Previous interventional stone treatments				
Open surgery		44	0.455 [0.215–1.894]	0.051
ESWL		154	1.474 [1.023–2.127]	0.063
PCNL		184	0.523 [0.358–0.754]	0.001
Guy's Stone Score				
1		192	1 (reference)	<0.001
2		270	0.279 [0.185–0.416]	<0.001
3		335	0.126 [0.083–0.188]	<0.001
4		164	0.081 [0.048; 0.132]	
Number of tracts				
1		729	1 (reference)	
2		182	0.314 [0.212–0.456]	<0.001
3 or more		50	0.404 [0.204–0.754]	0.006

BMI: body mass index; ESWL: external shock wave lithotripsy; PCNL: percutaneous nephrolithotomy.

as infundibular and calyx stenosis. Furthermore, the number of tracts and the GSS had a negative impact. Souza Melo et al.[17] validated and demonstrated that the GSS directly impacts surgery outcome of supine and prone PCNL and that the number of tracts may be related to the complexity of the case.

Multivariate analysis has shown importance of the GSS on success analysis. This nomogram can be easily used in preoperative evaluations, and it is quicker than S.T.O.N.E. score and CROES nomogram[20]. We also can use it to brief patients on postoperative results before the surgery. As GSS and the number of previous PCNL were predictive factors of success, we should be prepared for lower success rates in complex cases, and we must consider the use of other resources such as endoscopic combined intrarenal surgery (ECIRS)[21]. ECIRS is an important technique to increase success rates[22]. Regarding the antegrade flexible nephroscope use at the end of surgery, Gokce et al.[23] recently demonstrated that the retrograde approach may improve outcomes as more calyces can be reached and more fragments can be removed in this manner.

Comparing our success rates with previous results, we have obtained relatively poor results with our overall success rate at only 40.7% against the 75.7% of CROES PCNL global study[5]. This may be due to our high proportion of complex cases with only 31.69% of GSS 1. We have adopted the staged procedure for complex cases (GIII and GIV) to reduce complications. In these complex cases, we removed all pelvic stones for up to 90 min, lowered the middle pole, and left only the upper pole for the second procedure. If the patient is doing well within the 90-min duration, we continued the procedure. Many cases underwent a similar approach; therefore, it may be reflective of the relatively low success rate of a single procedure. Recently, Krambeck et al.[24] proved in a multi-institutional study, success rates on POD1 similar to ours (44.4%) with this approach. Furthermore, the use of CT on POD1 is a very rigorous criterion. We have decided to use CT, despite its radiation exposure, because of its precision in showing the immediate success rate and eventual complications. Moreover, in cases of residual stones, planning the next procedure will be necessary.

Ultrasound and kidney-urinary-bladder (KUB) imaging cannot demonstrate real success[5]. Antonelli et al.[9] compared CT with KUB and concluded that CT is the optimal post-PCNL imaging modality to detect RFs. It is also important to note that the CT scan can prematurely evaluate organ lesions. Some groups consider clinically insignificant fragments smaller than 2 mm; however, those smaller than 4 mm as RF, in accordance with Raman et al.'s study[1] that demonstrated that second-look flexible nephroscopy is not cost-efficient for RF ≤4 mm. The definition of stone-free status remains a point of debate. The evaluation of the patients on POD1 could provide lower numbers of stone-free patients even with the current definition since the RFs need some time to be expelled.

This retrospective study has limitations such as the problem of radiation exposure on performing CT on POD1 and the lower success rates. Nevertheless, we also want to highlight that this was a single-center study with a large number of patients wherein the standardized technique was employed, the consolidation of the use of GSS, and the importance of patient history in predicting the success with PCNL.

Therefore, it is important to note the use of GSS on preoperative evaluation, to advise patients on the success probability, and to expect lower success rates when the patient has previous PCNL. These observations may lead to technical improvement, as the use of retrograde nephroscopy at the end of the surgery has been a good option for checking patient status when being stone free is expected according to final fluoroscopy.

CONCLUSION

GSS and the number of previous PCNL are predictors of success with the supine position. Complex cases and with previous percutaneous interventions may require technical improvements to achieve higher stone-free rates.

AUTHORS' CONTRIBUTIONS

KKRH: Data curation, Formal analysis, Investigation, Writing – original draft. **RP:** Conceptualization, Data curation, Formal analysis, Methodology, Project administration, Writing – original draft. **DBF:** Data curation, Formal analysis, Software. **CAB:** Data curation, Methodology. **PKVM:** Data curation. **DJC:** Data curation. **CBM:** Data curation, Project administration, Visualization. **JFAC:** Supervision. **FCV:** Conceptualization, Data curation, Formal analysis, Methodology, Project administration, Supervision, Writing – original draft.

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Polymorphisms rs2010963 and rs833061 of the VEGF gene in polycystic ovary syndrome

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SUMMARY

OBJECTIVE: The polycystic ovary syndrome is the most common endocrine disorder, characterized by the dysregulation of ovarian angiogenesis. This alteration can be related to changes in the activities of the vascular endothelial growth factor (VEGF) gene. Single-nucleotide polymorphisms have been observed in the promoter, intronic, and untranslated regions of the VEGF gene, and several studies have suggested that these polymorphisms may be associated with the risk of polycystic ovary syndrome. This study aimed to investigate the association between rs2010963 and rs833061 polymorphisms and haplotypes of VEGF in the etiology of polycystic ovary syndrome.

METHODS: A total of 210 women, 102 diagnosed with polycystic ovary syndrome and 108 controls, participated in this study. The genotyping of the samples was performed by PCR-RFLP and real-time PCR for rs2010963 and rs833061 polymorphisms, respectively. The statistical analyses were performed by the chi-square test and logistic regression model.

RESULTS: The clinical characteristics of the patients showed that 75.8% of the patients did not become pregnant, 36.3% had a family history of polycystic ovary syndrome, 58.6% were obese, and about 60% had clinical characteristics of hyperandrogenism. There were no associations between the distribution of rs2010963 (OR 1.24; 95%CI 0.60–2.57; p=0.56) and rs833061 (OR 0.78; 95%CI 0.32–1.92; p=0.59) in patients and controls.

CONCLUSIONS: The patients with polycystic ovary syndrome have similar rates of VEGF polymorphisms rs2010963 and rs833061 on the general population.

KEYWORDS: Polycystic ovary syndrome. Polymorphism, genetic. Haplotypes. Vascular endothelial growth factor A.

INTRODUCTION

Polycystic ovary syndrome (PCOS) is the most common endocrine disorder, and its clinical features include hirsutism, infertility, acne, alopecia, oligo-anovulation, and metabolic abnormalities such as insulin resistance, excessive weight or obesity, type 2 diabetes, dyslipidemia, and an increased risk of cardiovascular disease[1,2].

Dysregulation of ovarian angiogenesis contributes to abnormal follicular development in women with PCOS. This alteration may contribute to the ovarian features of PCOS, such as abnormal follicular development, increase in the number of small follicles, and failure in the selection of the dominant follicle, with anovulation and cyst formation[3].

Modifications in the vascular endothelial growth factor (VEGF) family are associated with the ovarian angiogenesis[3]. In the ovary, this gene is expressed in theca cells, granulosa lutein cells, and interstitial tissues and may be involved in the physiological regulation of ovarian angiogenesis, in a manner that suggests a role of this growth factor in both cyclic angiogenesis and regulation of vascular permeability, both critical for ovarian folliculogenesis and for normal reproductive function[4]. The VEGF gene is located in the chromosome region 6p21.3[5]. Single-nucleotide polymorphisms (SNPs) have been observed in the promoter, intronic, and untranslated regions of the VEGF gene, and several studies have suggested VEGF gene polymorphisms may be associated with PCOS risk[6-8].

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VEGF rs833061 T and rs2010963 G alleles appear to correlate with altered VEGF expression levels. The increased levels of VEGF have been reported in PCOS6. The rs2010963 and rs833061 SNPs VEGF have recently been investigated in five independent studies6-10. One of them evaluated the association of VEGFA SNPs (nine tested variants) with altered VEGF secretion level and PCOS among ethnically matched control women. This study showed that VEGF levels in rs833061 genotypes were significantly higher in PCOS9. The other study also published in 2019 investigated SNPs rs2010963 and rs833061 and showed that the first one may be associated with the risk of PCOS in Chinese women10. The studies published in 20206-8 are of the meta-analysis type and confirm associations of polymorphisms in the VEGF gene with susceptibility to PCOS, with emphasis on rs20109637,8.

The rs2010963 polymorphism had been described as C→T exchange at nucleotide position 936, in the 3′-UTR of the VEGF gene, and was associated with lower VEGF plasma levels[11]. Therefore, the rs833061 is located in the promoter region and has been associated with increased VEGF expression levels[12].

In the literature, there are several studies on polymorphisms in the VEGF gene in patients with PCOS from different populations; however, no research was conducted on these two polymorphisms in Brazilian women.

The objective of this study was to investigate the association of the VEGF polymorphisms rs201093 and rs833061 and to identify the frequency of haplotypes with the risk of developing PCOS in women compared with control group.

METHODS

Subjects

This study was approved by the Research Ethics Committee of the Federal University of Triângulo Mineiro (UFTM), protocol 1796, and all participants signed an informed consent form.

Participants' inclusion in the study occurred in the period from 2012 to 2016. This case-control study included 102 patients with a clinical diagnosis of PCOS and 108 control women. The PCOS diagnosis was based on Rotterdam criteria, and the patients who visited the Endocrinology and Gynecology Outpatient Clinic of the UFTM were selected. In the control group, women at reproductive age who had no history of hyperandrogenism, menstrual dysfunction, infertility, or sonographic sign of PCOS, and those who sought medical care for gynecological routine were selected for the study. All participants answered a questionnaire about the risk factors.

Molecular analysis

Genomic DNA from the peripheral blood was extracted by a salting-out method.

Genotyping of the rs2010963 polymorphism was performed in 106 PCOS patients and 97 controls using polymerase chain reaction restriction fragment length polymorphism (PCR-RFLP) analysis.

The PCR was carried out in a total volume of 30 µL containing approximately 100 ng genomic DNA, 1× PCR buffer, 1 mM $MgCl_2$ (25 mM), 2 µM of dNTP, 20 pmol of each primer (F: 5′-CCGACGGCTTGGGGAGATTG-3′; R: 5′-CGGCGGTCACCCCCAAAAG-3′), 1 U of Taq DNA polymerase, and 5% of glycerol. The amplification program consisted of an initial denaturation step at 94°C for 10 min and 40 cycles (denaturation at 94°C for 45 s, annealing for 45 s at 62°C, extension at 72°C for 30 s) and final extension for 10 min at 72°C.

The PCR products were digested with 0.2 U of the BsmFI restriction enzyme. Digested fragments were analyzed by 10% polyacrylamide gel electrophoresis. After the restriction enzyme treatment, the CC genotype was visualized as a single 197 bp fragment; the CG genotype as three fragments of 197, 167, and 30 bp; and the GG genotype was visualized as two fragments of 167 and 30 bp.

The real-time PCR allelic discrimination technique was used to analyze the rs833061 polymorphism in 94 patients and 87 controls, on the ABI PRISM 7500 Sequence Detection System (Applied Biosystems) using TaqMan Minor Groove Binder (MGB) probes. Primers and probes were designed by Life Technologies (ID: C_1647381_10).

Statistical analysis

The chi-square test was used for the statistical analysis of the genotypic and allelic distribution of the polymorphisms, as well as to verify the Hardy-Weinberg equilibrium (HWE). A statistical power of 95% was tested using the G* Power program 3.1.9.2. In addition, a post-hoc test with the total sample (n=210) was performed, with an effect size of 0.27 and an alpha significance level of 0.05. The multiple logistic regression model was used to determine the effect of risk factors (e.g., family history, smoking, alcohol consumption, and the presence of polymorphism) in PCOS. The SNPStats program was used for the logistic regression model adjusted for age. The

effect of polymorphisms was evaluated by the following inheritance models: codominance, dominance, recessive, and overdominance.

The haplotype from VEGF gene polymorphisms was inferred using the SNPStats program, checking the estimated population frequency of the haplotypes.

The results were presented in the odds ratio (OR) and 95% confidence interval (95%CI), with the level of statistical significance being defined as $p<0.05$. A post-hoc analysis was performed, and the statistical power for association tests was found to be 98%.

RESULTS

The clinical characteristics of the patients showed that 75.8% of the patients did not become pregnant, 36.3% had a family history of PCOS, 58.6% were obese, and about 60% had clinical characteristics of hyperandrogenism.

In the univariate analysis, no statistically significant differences were observed between the two groups for polymorphisms rs2010963 and rs833061 (χ^2=0.38, p=0.83 and χ^2=0.86, p=0.65, respectively). For the allele frequencies, no differences were found between the groups (χ^2=0.18, p=0.67 and χ^2=0.02, p=1.00, respectively).

The genotype distributions of the rs2010963 and rs833061 polymorphisms were in Hardy-Weinberg equilibrium in both patient (χ^2=2.35; p=0.12; χ^2=0.05; p=0.82) and control (χ^2=1.05; p=0.31; χ^2=1.04; p=0.31) groups.

The genotypes of 90 women with PCOS and 80 controls were adjusted for age according to the heritable models and showed no association between the polymorphisms and PCOS (Table 1).

The haplotypes that were constructed with the analysis of the two VEGF gene polymorphisms were evaluated in this study. All estimated haplotypes had similar frequencies between the two groups.

Table 1. Association of rs2010963 and rs833061 polymorphisms of the vascular endothelial growth factor gene with polycystic ovary syndrome, adjusted for age (used in case-control study).

Model	Genotype	Case n (%)	Control n (%)	OR (95%CI)	p
rs2010963					
Codominance	C/C	29 (32.2)	27 (33.8)	1.00	0.88
	C/G	40 (44.4)	36 (45.0)	1.16 (0.56–2.41)	
	G/G	21 (23.3)	17 (21.2)	0.98 (0.41–2.35)	
Dominance	C/C	29 (32.2)	27 (33.8)	1.00	0.78
	C/G-G/G	61 (67.8)	53 (66.2)	1.10 (0.56–2.17)	
Recessive	C/C-C/G	69 (76.7)	63 (78.8)	1.00	0.78
	G/G	21 (23.3)	17 (21.2)	0.90 (0.42–1.92)	
Overdominance	C/C-G/G	50 (55.6)	44 (55.0)	1.00	0.62
	C/G	40 (44.4)	36 (45.0)	1.17 (0.62–2.22)	
rs833061					
Codominance	C/C	30 (33.3)	25 (31.2)	1.00	0.58
	C/T	44 (48.9)	43 (53.8)	1.32 (0.65–2.68)	
	T/T	16 (17.8)	12 (15.0)	0.86 (0.32–2.27)	
Dominance	C/C	30 (33.3)	25 (31.2)	1.00	0.62
	C/T-T/T	60 (66.7)	55 (68.8)	1.19 (0.60–2.34)	
Recessive	C/C-C/T	74 (82.2)	68 (85.0)	1.00	0.47
	T/T	16 (17.8)	12 (15.0)	0.73 (0.30–1.73)	
Overdominance	C/C-T/T	46 (51.1)	37 (46.2)	1.00	0.32
	C/T	44 (48.9)	43 (53.8)	1.38 (0.73–2.61)	

OR: odds ratio; CI: confidence interval. Significant $p<0.05$.

The multiple logistic regression data are shown in Table 2, considering the risk factors (e.g., family history, smoking, and alcoholism) and the two polymorphisms studied in PCOS patients (n=88) and controls (n=81). It was evidenced that the family history is more frequent in patients with PCOS, smoking is more frequent in controls, and there are no differences in relation to alcoholism and in the distribution of the rs2010963 and rs833061 polymorphisms.

DISCUSSION

VEGF alterations characterize numerous pathologies, either with increased, decreased, or abnormal angiogenesis. Therefore, it has been suggested that these alterations may be associated with the decreased, or lack of, ovulation rates and with the formation of many antral follicles in the PCOS ovaries. According to the literature, further studies are required to clarify the role of angiogenesis in PCOS and to develop new potential therapies[2,3], such as bromocriptine, metformin, and melatonin[13-15].

The VEGF is the main angiogenic factor that promotes endothelial cell proliferation and migration and vascular permeability[3]. Thus, genetic analysis in the VEGF gene may help clarify the pathogenesis of PCOS.

To the best of our knowledge, this is the first molecular study to investigate the association between rs2010963 and rs833061 polymorphisms and PCOS susceptibility in Brazilian women. With regard to VEGF, our group evaluated the polymorphisms rs3025039, rs1570360, and rs699947 and showed that the polymorphism rs1570360 is associated with PCOS and that the T-G-C haplotype could be associated with protective factors[16].

Several VEGF SNPs are associated with various conditions in women, including endometriosis[17,18], recurrent miscarriage[19], and preeclampsia[20]. These results together suggest that VEGF SNP can contribute to the pathogenesis of female reproductive diseases.

In the sample analyzed, the rs2010963 and rs833061 polymorphisms are not associated with PCOS. The lack of significance in this study may be due to the sample size, sample stratification, and ethnic issues related to the Brazilian population. However, they have been extensively studied with conflicting results (Table 3)[9,10,12,21-24], probably due to different ethnicities. It is worth mentioning that PCOS is a multifactorial disease and environmental factors and polymorphisms in genes other than VEGF might play an important role in women's susceptibility to its occurrence.

Table 2. Distribution of the polymorphisms rs2010963 and rs833061 of vascular endothelial growth factor gene and risk factor in cases (n=88) and controls (n=81).

Evaluated variable	Cases n (%)	Controls n (%)	OR (95%CI)	p
Smokers				
Yes	07 (7.9)	22 (27.2)	0.20 (0.08–0.55)	**<0.05**
No	81 (82.1)	59 (72.8)		
Alcoholism				
Yes	21 (23.9)	22 (27.2)	1.11 (0.51–2.41)	0.79
No	67 (76.1)	59 (72.8)		
Family history of PCOS				
Yes	35 (39.8)	17 (21.0)	2.70 (1.30–5.62)	**<0.05**
No	53 (60.2)	64 (79.0)		
rs2010963				
CC	61 (69.3)	27 (33.3)	1.24 (0.60–2.57)	0.56
CT/TT	27 (30.7)	54 (66.7)		
rs833061				
CC	16 (18.2)	12 (14.8)	0.78 (0.32–1.92)	0.59
CT/TT	72 (81.8)	69 (85.2)		

OR: odds ratio; CI: confidence interval; PCOS: polycystic ovary syndrome. Bold value indicates significant p<0.05.

Table 3. Summary of the mains results of previous studies that investigated rs2010963 and rs833061 single-nucleotide polymorphisms of vascular endothelial growth factor gene in polycystic ovary syndrome.

Study	VEGF gene polymorphisms	Sample	Main results and/or conclusions
Bao et al.[9]	rs1547651, rs1570360, rs2010963, rs3025020, rs3025039, rs699947, rs833061, rs833058, and rs833068	55 women with PCOS and 52 control subjects.	VEGF levels in rs699947 (AA-major homozygous), rs3025039 (CC-major homozygous), and rs833061 (TT & CC-major & minor homozygous) genotypes were significantly higher in PCOS. The study results evidently proved that the allelic variants in genes may be a factor for PCOS and VEGF serum levels with respect to few SNP variants only.
Huang et al.[10]	rs2010963, and rs833061	118 women with PCOS and 130 controls.	Our study shows for the first time that the rs2010963 polymorphism may be associated with a risk of PCOS in Northern Chinese women.
Almawi et al.[21]	rs833052, rs1547651, rs699947, rs833061, rs1570360, rs2010963, rs25648, rs833068, rs833070, rs3025020, rs3025026, and rs3025039	382 women with PCOS, and 393 control subjects.	Among the 12 tested VEGFA SNPs, minor allele frequency of only rs3025020 was significantly higher in PCOS cases than control women. Increased and reduced PCOS risk was seen with rs3025020 and rs2010963 genotypes, respectively. Increases and reduction in VEGF levels were associated with rs3025020 and rs2010963, respectively. Our study also confirmed the association of CAACAGCGA haplotype with increased risk of PCOS.
Ben Salem et al.[22]	rs699947, rs833061, rs1570360, rs833068, rs3025020, and rs3025039	118 PCOS patients and 150 controls.	We observed 10 haplotypes in our studied cohort where H1 (ACGG), H2 (ACAG), H7 (CTGG), and H8 (CTGA) were the most frequent. We observed the association of the genotype CT of the SNP rs3025039 with PCOS phenotype.
Guruvaiah et al.[23]	-460C/T and +405C/G	126 PCOS patients and 130 controls.	The frequencies of +405G/G genotype (p=0.03) and +405G alleles (p=0.006) were significantly higher in patients compared to controls. Whereas the genotype and allele frequencies of -460C/T SNP were not significantly different between patients and controls. Our findings suggest that the VEGF +405C/G polymorphism may constitute an inheritable risk factor for PCOS in South Indian women.
Vural et al.[12]	-2578A/C, -460T/C, and +405C/G	137 patients with PCOS and 155 healthy women.	We did not find any evidence for association between PCOS and the three individual SNPs. The haplotype -2578C/-460T/+405G was significantly overexpressed in the PCOS group in comparison with controls (p=0.019).
Lee et al.[24]	-2488C>T, -634G>C, -7C>T, +3436G>C, +6112C>A, +6594C>T, +9374G>A, +9812C>T, +13125C>T, and +13553C>T	134 patients with PCOS and 100 healthy women as controls.	We concluded that one novel SNP at +9812 site, one known SNP at +13553 site, and one selected haplotype in the ***VEGF*** gene have a high possibility of significant associations with the pathogenesis of PCOS in a Korean population.

There are seven studies that investigated the polymorphisms rs2010963 and rs833061 of the VEGF gene. Three studies did not confirm any association between PCOS and the polymorphisms investigated[17,19,20], a result similar to that found in this study. In contrast, other studies found an association of polymorphism rs2010963[17,22,23] and rs833061[10] with PCOS.

In relation to haplotype analysis, two studies showed a relationship between haplotypes and PCOS[21,24]. However, there is no significant difference in the occurrence of the four haplotypes between the controls and cases, in relation to the polymorphisms rs2010963 and rs833061[17], which is in agreement with our results.

A meta-analysis of seven studies showed there is little association between PCOS risk and the VEGF gene polymorphisms rs2010963, rs833061, and rs699947 in the general populations, whereas the genotype CC (rs2010963) might decrease the risk of PCOS among Asian women[8]. Another recent meta-analysis included 10 relevant case-control studies, involving 1347 PCOS cases and 1378 controls. The VEGF rs2010963 polymorphism was associated

with decreased PCOS risk in the whole population and the Asian populations[9].

This study demonstrated that family history is more frequent in patients with PCOS. According to the literature, multiple familial and twin studies confirmed the role of genetics in the etiology of PCOS with high heritability of 70%[21].

There are some limitations to our study. The serum levels of the VEGF are not measured. However, it is also worth mentioning that our study so far is the only one to evaluate these polymorphisms in the Brazilian population.

CONCLUSIONS

The PCOS patients have similar rates of VEGF polymorphisms rs2010963 and rs833061 on the general population.

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Evaldo Maia collected blood samples.

AUTHORS' CONTRIBUTIONS

ALSAV: Data curation, Writing – original draft. **ABTM:** Data curation, Writing – review & editing. **MKOG:** Methodology, Writing – review & editing. **AVAL:** Methodology, Writing – review & editing. **MASB:** Methodology, Writing – review & editing. **SCSVT:** Methodology, Writing – review & editing. **EAMRR** Methodology, Writing – review & editing. **MFPL:** Methodology, Writing – review & editing. **MTRC:** Conceptualization, Data curation, Formal analysis, Writing – original draft, Writing – review & editing, Supervision, Project administration.

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ORIGINAL ARTICLE

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Serum Prealbumin: a potential predictor of Right Ventricular Dysfunction in patients receiving programmed hemodialysis

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SUMMARY

OBJECTIVE: Prealbumin has been a reliable marker to predict protein energy malnutrition and hypercatabolic state. In this analysis, we particularly aimed to investigate the potential association between serum prealbumin levels and right ventricular dysfunction in patients receiving programmed hemodialysis.

METHODS: A total of 57 subjects were included in the analysis. The subjects were then categorized into two groups: right ventricular dysfunction (n=18) and non-right ventricular dysfunction (n=39) groups. In all patients, detailed transthoracic echocardiography (following hemodialysis) were performed along with the evaluation of complete blood count, routine biochemistry parameters, and, in particular, serum prealbumin levels.

RESULTS: Mortality rate at 3 years was found to be significantly higher in the right ventricular dysfunction group (p=0.042). Serum prealbumin levels were also significantly lower in the right ventricular dysfunction group compared with the non-right ventricular dysfunction group (23.83±8.50 mg/dL versus 31.38±6.81 mg/dL, p=0.001). In the receiver operating characteristics curve analysis, a prealbumin cutoff value of <28.5 mg/dL was found to predict right ventricular dysfunction, with a sensitivity of 67% and a specificity of 62% (area under the curve: 0.744). In the correlation analysis, a moderate yet significant positive correlation was demonstrated between serum prealbumin and tricuspid annular plane systolic excursion (r=0.365, p=0.005).

CONCLUSION: This study suggests that low serum prealbumin might serve as a potential predictor of right ventricular dysfunction (and its clinical consequences) in patients receiving programmed hemodialysis.

KEYWORDS: Hemodialysis. Malnutrition. Prealbumin. Mortality. Right ventricular dysfunctions.

INTRODUCTION

Chronic renal failure (CRF) is generally regarded as a progressive loss of nephron mass in an irreversible manner[1] and might potentially result in end-stage renal failure (ESRF) after a certain period. In contrast, ESRF inevitably necessitates life-saving renal replacement therapies, including renal transplantation, peritoneal dialysis, and hemodialysis (HD)[1]. In particular, malnutrition is commonly encountered in patients receiving programmed HD (mild-to-moderate and severe malnutrition in 33% and 6–8% of patients, respectively)[2]. Malnutrition has also been a common problem in heart failure (HF) and has a strong link with unfavorable outcomes[3]. Moreover, malnutrition in patients with CRF might lead to a variety of cardiovascular complications that are held responsible for increased morbidity and mortality[4]. To date, left ventricular (LV) functions have been the focus of interest in patients with CRF. However, implications of right ventricular dysfunction (RVD) remains to be further established in these patients[5]. Interestingly, HD might significantly increase the risk of RVD largely due to the hemodynamic impact of brachial arteriovenous fistula that leads to a state of left-to-right shunt with consequent increases in cardiac preload[6].

Clinically, there have been many methods to evaluate malnutrition in HD patients. In this setting, assessment of weight loss, anorexia, vomiting, body mass index, upper arm muscle circumference, and handgrip strength might aid in the gross evaluation of malnutrition in these patients. Moreover, certain biochemical parameters including albumin, prealbumin, transferrin, and insulin-like growth factor might more objectively predict an existing malnutrition[7]. Specifically, prealbumin has been regarded as a marker of nutritional and inflammatory status, and accordingly, low serum levels of this marker might be associated with unfavorable outcomes in the setting of HF[7]. Importantly, RVD has been recently suggested as a predictor of cardiovascular death, both in the settings of HF and programmed HD[8].However, the association of prealbumin with

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RVD in the setting of CRF remains to be elucidated. In this study, we aimed to focus on the potential link between serum prealbumin and RVD in patients receiving programmed HD.

METHODS

The study comprised a total of 57 patients who were categorized into two groups: patients with RVD (RVD group) and those without RVD (non-RVD group). RVD and non-RVD groups were composed of 18 and 39 patients, respectively. Particular inclusion criteria were as follows: the need for a programmed HD, being at the age of >18 years, having a history of programmed HD in a dialysis center three times a week for at least 3 months, and having no active infection, malignancy, or left HF. In contrast, patients with secondary causes of RVD (including chronic obstructive pulmonary disease, morbid obesity, pulmonary hypertension [primary and secondary forms], and pulmonary thromboembolism) were excluded from the study. The subjects were under regular clinical follow-up on an annual basis. Annual clinical, laboratory (e.g., prealbumin and C-reactive protein [CRP]), and transthoracic echocardiographic (TTE) examinations were regularly filled in follow-up forms. Thereafter, the results were statistically analyzed. In particular, the potential relationship between RVD and serum prealbumin levels was investigated in these patients. The study protocol conformed to the Declaration of Helsinki and was endorsed by the local Ethics Committee. The committee waived the informed consent due to the retrospective nature of the study.

In all patients, TTE and Doppler echocardiographic examination were performed with a 3.5-MHz transducer. Calculation of left ventricular ejection fraction (LVEF) was performed on apical two- and four-chamber views using the modified Simpson's method. Evaluation of RV functions was performed based on tricuspid annular plane systolic excursion (TAPSE), fractional area change (FAC), and E/E′. RVD was defined as a TAPSE value of ≤15 mm[9]. Patients with severe tricuspid regurgitation were also excluded as the evaluation of RV systolic functions by TAPSE method might not be proper in the setting of severe tricuspid regurgitation.

Statistical analysis

All statistical analyses were done by using SPSS software (IBM SPSS Statistics for Windows, version 21.0; Armonk, NY, USA). Continuous variables with a normal distribution were expressed as mean±standard deviation, and variables with non-normal distribution were expressed as median (interquartile range). Categorical variables were presented as number (percentage). Statistical analysis between the two groups involved Student's t-test for normal data and Mann-Whitney U test for non-normal data. Categorical variables were compared by chi-square test. Pearson's correlation was used for analyzing correlation between serum prealbumin and TAPSE. Receiver operating characteristic (ROC) curve was utilized to evaluate the cutoff value for serum prealbumin in predicting RVD. A p-value of <0.05 was considered statistically significant.

RESULTS

Baseline characteristics of both groups are presented in Table 1. There were no significant diversities between the two groups in terms of age; gender; duration of HD; body mass index; serum levels of phosphate, aluminum, magnesium, uric acid, albumin, creatinine, and lipid parameters;

Table 1. Comparison of the two groups with regard to baseline clinical and laboratory data.

Variable	Right ventricular dysfunction		p-value
	No (n=39)	Yes (n=18)	
Age (years)	59.7±13.1	64.4±15.2	0.240
Male gender, n (%)	23 (59.0)	9 (50.0)	0.526
Duration of hemodialysis (year)	5 (2-8)	6 (3–9)	0.356
Hypertension, n (%)	22 (56.4)	9 (50.0)	0.625
Diabetes mellitus, n (%)	15 (38.4)	7 (38.8)	0.972
Active smoking, n (%)	16 (41.0)	9 (50.0)	0.728
BMI (kg/m^2)	23.9±2.9	25.1±7.9	0.469
Serum albumin (mg/dL)	3.80±0.41	3.77±0.38	0.810
Serum creatinine (mg/dL)	7.44±1.50	6.97±2.56	0.437
Total cholesterol (mg/dL)	166±34	165±20	0.898
LDL cholesterol (mg/dL)	103±27	100±18	0.692
HDL cholesterol (mg/dL)	34±11	40±11	0.100
Triglyceride (mg/dL)	176±96	158±78	0.549
Serum prealbumin (mg/dL)	31.38±6.81	23.83±8.50	0.001
Serum uric acid (mg/dL)	5.33±0.76	5.51±0.95	0.495
Serum aluminum (mg/dL)	15.6±9.7	15.2±7.2	0.882
Serum magnesium (mg/dL)	2.23±0.27	2.14±0.29	0.327
Serum phosphate (mg/dL)	5.11±1.33	4.85±1.26	0.216
Serum CRP (mg/dL)	0.80 (0.40–1.60)	1.45 (0.95–3.30)	0.023
Mortality for 3 years follow-up, n (%)	3 (7.7)	5 (27.8)	0.042

BMI: body mass index; LDL: low-density lipoprotein; HDL: high-density lipoprotein; CRP: C-reactive protein.

and histories of diabetes, hypertension, and active smoking. Serum CRP levels appeared to be higher in the RVD group compared with the non-RVD group (p=0.023). Moreover, mortality at 3 years was also significantly higher in the RVD group (p=0.042). In particular, serum prealbumin levels appeared to be lower in the RVD compared with the non-RVD group (23.83±8.50 mg/dL versus. 31.38±6.81 mg/dL, p=0.001) (Figure 1).

Echocardiographic parameters including pulmonary artery systolic pressure (PASP), TAPSE, E/E′ ratio, and FAC values also significantly differed between the groups. RVD group had higher values of PASP and E/E′ ratio (p=0.001 and 0.034, respectively) along with lower values of TAPSE and FAC (p<0.001 for both) (Table 2). In the ROC curve analysis, a prealbumin cutoff value of <28.5 mg/dL was found to predict RVD with a sensitivity of 67% and a specificity of 62% (area under the curve: 0.744) (Figure 2).

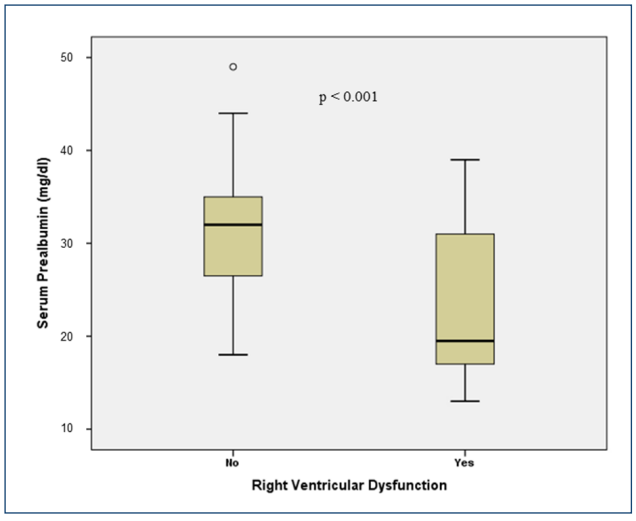

Figure 1. Comparison of serum prealbumin levels between the two groups.

Table 2. Comparison of the two groups with regard to baseline echocardiographic parameters.

	Right ventricular dysfunction		p-value
	N (n=39)	Yes (n=18)	
LVEF (%)	60±5	58±9	0.138
Right ventricular function parameters			
TAPSE	21.3±4.2	12.8±1.9	<0.001
Fractional area change (%)	44.1±6.3	33.4±8.6	<0.001
Left atrial volume (mL)	145±56	130±44	0.358
E/E′ ratio	16±6	22±7	0.034
PASP (mmHg)	30.9±4.9	37.7±9.2	0.001

LVEF: left ventricular ejection fraction; PASP: pulmonary artery systolic pressure; TAPSE: tricuspid annular plane systolic excursion.

The correlation analysis exhibited a moderate positive correlation between serum prealbumin and TAPSE values (r=0.365, p=0.005) (Figure 3).

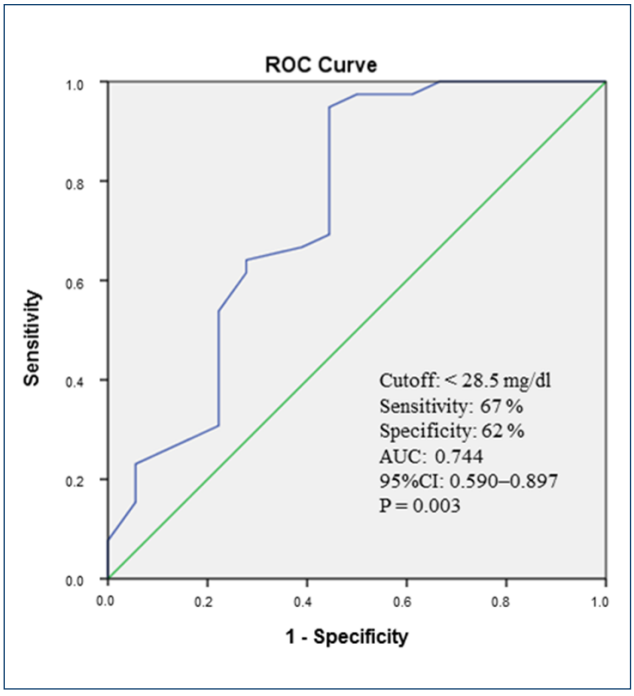

Figure 2. Receiver operating characteristics curve analysis of prealbumin in the prediction of right ventricular dysfunction.

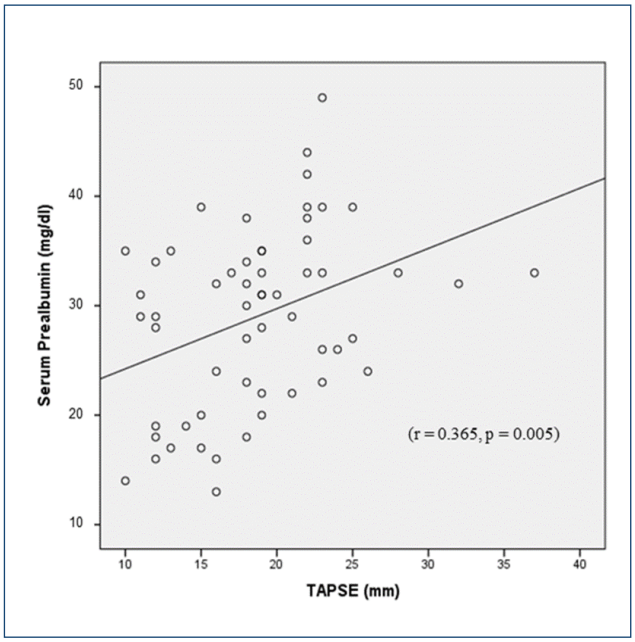

Figure 3. Pearson's correlation analysis between serum prealbumin and tricuspid annular plane systolic excursion.

DISCUSSION

In the present analysis, we have demonstrated a significant association between low serum prealbumin levels (a marker of malnutrition) and RVD (a predictor of low quality of life with consequent mortality) in a population of HD patients.

To date, two major types of malnutrition have been described in patients with CRF. The first type is characterized by low serum albumin levels possibly attributable to the reduced intake of energy and protein as a consequence of uremic toxicity. The second type generally presents with low prealbumin levels due to protein catabolism, increased resting energy expenditure, and significant increments in oxidative stress and pro-inflammatory cytokine levels (e.g., CRP)[10]. In HD patients, these two types are typically observed in combination[11]. Of note, emerging hyperinflammatory state generally marks the initiation of complications in HD patients. This hyperinflammation is largely attributable to the associated uremia that elicits a significant imbalance between pro-inflammatory and anti-inflammatory milieu largely through induction of pro-inflammatory cytokine production[12]. Accordingly, CRP levels are well known to be high in these patients, potentially leading to an increased cardiovascular mortality as well[13]. Specifically, we were able to demonstrate even higher levels of serum CRP in HD patients with RVD. Importantly, this suggests that an existing RVD might have important implications in the generation of a more substantial inflammatory response in HD patients. However, the issue of whether RVD serves as the cause or consequence (or both) of hyperinflammation still needs to be established in this context.

In contrast, cardiac cachexia is well known to be associated with malnutrition and systemic inflammation in patients with HF[14]. Furthermore, cytokines including interleukin-6 might not only alter intestinal permeability and elicit cardiac cachexia but also have an important pathogenetic role in the genesis and perpetuation of HF[14]. In the specific context of RVD, PASP and endothelial dysfunction were previously suggested to be associated with RVD[15,16]. More specifically, RVD has gained a significant reputation as a predictor of cardiovascular mortality both in the settings of HF and HD[8]. As previously mentioned, lower levels of prealbumin (a marker of nutritional and inflammatory status) appear to have a strong link with unfavorable outcomes in the setting of HF[7]. Prealbumin has also been shown to be associated with nutrition and inflammation in HD patients[17]. Notably, prevalence of RVD is significantly higher in HD patients and is regarded as the fundamental cause of mortality in patients with ESRF[18]. In this study, we have uncovered the potential link between low prealbumin levels (that indirectly denotes malnutrition) and RVD in HD patients. Furthermore, rate of mortality appeared to be substantially higher in patients with RVD at 3 years. This increased mortality might not only be due to the direct consequences of right HF (e.g., arrhythmogenesis and progressive hypoperfusion) but also be strongly associated with the underlying hyperinflammatory state. In this context, systemic inflammation is well known to be associated with a variety of cardiovascular events, including induction of acute coronary syndromes and malignant arrhythmogenesis[19]. In this setting, evaluation of serum prealbumin may potentially help predict an existing RVD and its clinical consequences in HD patients. This might also enable to implement patient-specific management strategies in an effort to improve overall prognosis.

Study limitations

The relatively small cohort of patients possibly arises as the most significant limitation. The utility of afterload-dependent parameters including TAPSE might be regarded as another limitation. However, this challenge was partially mitigated through acquisition of calculations right after HD. Moreover, the mean TAPSE values were calculated by two cardiologists to enhance the objectivity of method. More importantly, the diagnostic power of prealbumin for the prediction of RVD was only moderate. However, it might possibly have a higher predictive value in larger cohorts. Finally, we were not able to evaluate other inflammation markers that might also have important implications in this setting.

CONCLUSIONS

In patients receiving programmed HD, prealbumin (an indirect indicator of malnutrition) may potentially predict an existing RVD that might be associated with unfavorable outcomes. Therefore, serum prealbumin, as an important prognostic marker, may be evaluated at regular intervals in these patients.

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AUTHORS' CONTRIBUTIONS

MG: Data curation, Methodology, Writing – original draft, Writing – review & editing. **AK:** Data curation, Methodology, Writing – review & editing. **GT:** Data curation, Methodology **EIS:** Data curation, Software, Validation, Writing – original draft. **KY:** Data curation, Writing – original draft, Writing – review & editing.

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ORIGINAL ARTICLE

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Potentially inappropriate medication use in hospitalized elderly patients

Regina Maria Alexandre Fernandes de Oliveira[1*], Milton Luiz Gorzoni[2], Ronaldo Fernandes Rosa[2]

SUMMARY

OBJECTIVE: This study aimed to assess the prevalence of potentially inappropriate medication prescription in hospitalized elderly patients according to the 2019 American Geriatrics Society Beers Criteria.

METHODS: This study is a prospective analysis of electronic medical records of elderly patients admitted to the Department of Medicine, Hospital Central da Irmandade da Santa Casa de Misericórdia de São Paulo, between 1 September 2020 and 30 April 2021.

RESULTS: A total of 142 patients (85 women and 57 men) with a mean age of 74.5±7.3 years (65–99 years) were assessed. Of these, 108 (76.1%) were elderly (age ≥65 years and <80 years) and 34 (23.9%) long-lived (age ≥80 years). The average length of stay found in the sample was 25.3±28.7 days (between 2 and 235 days), and 102 out of the 140 patients assessed remained in the hospital for up to 29 days. Sixteen drugs considered potentially inappropriate medication were found in the patients' prescriptions, with at least one potentially inappropriate medication having been prescribed to 141 (99.3%) patients. Elderly patients had a mean of 2.57±0.94 potentially inappropriate medication prescribed versus 2.56±0.89 among long-lived patients. The most prescribed potentially inappropriate medication were as follows: regular human insulin as required (85.2%), and omeprazole (73.9%) and metoclopramide as required (61.3%).

CONCLUSION: The study sample showed significant percentages of potentially inappropriate medication prescriptions for the elderly admitted to the hospital.

KEYWORDS: Potentially inappropriate medication list. Inpatients. Side effects. Hospitalization. Aged. Iatrogenic disease. Inappropriate prescribing.

INTRODUCTION

The number of elderly people and life expectancy worldwide have both increased significantly. This characterizes the phenomenon of aging, in which the population aged 65 years and older grows at a rate of about 3% per year, a rate that is higher than those in any other age group[1,2].

Aging consists of a progressive inability to maintain the homeostatic balance and is associated with the decline of organic functions, which results in a predisposition of the elderly population to develop multiple comorbidities. In turn, the direct consequence of this scenario is a higher prevalence of older adults being hospitalized, in which a wide variety of drugs are used in addition to those chronically used by them[3,4].

The elderly population, however, has a number of age-specific conditions that influence drug metabolism and pharmacokinetics thereof. Thus, both first-pass metabolism and hepatic clearance can be altered, which increases the bioavailability of xenobiotics in the elderly. Furthermore, changes in body composition occur with age, and lipophilic drugs may have a greater distribution volume with a longer half-life, whereas hydrophilic drugs have a lower distribution volume. Finally, renal function is globally reduced, as the vast majority of elderly people have some degree of renal dysfunction[5,6].

In this context, the concept of potentially inappropriate medication (PIM) use in the elderly must be taken into account[7], and the use thereof represents greater risks of causing adverse reactions to patients due to the changes inherent to aging. Therefore, lists have been created in order to assist clinical practitioners in identifying PIM and preventing their prescription[8-10].

The so-called "Beers criteria" of the American Geriatrics Society (AGS), developed by an American team of specialists, comprising, among others, geriatricians, pharmacologists, and clinical pharmacists, is one of those lists that are readily

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available and has become the most cited and used worldwide for detecting PIM[11,12].

Refraining from using PIM in the elderly is an important public health strategy, since it optimizes the appropriate prescription for this population, thereby preventing potentially negative outcomes such as predictable adverse drug reactions, prolonged hospital stay, disabilities, and death[13,14].

The goal of this study was to assess the prevalence of PIM use in hospitalized elderly patients according to the 2019 AGS Beers criteria[15].

METHODS

Study design

This is a prospective analysis of electronic medical records of elderly patients admitted to the Department of Medicine at Hospital Central da Irmandade da Santa Casa de Misericórdia de São Paulo (ISCMSP) between September 1, 2020 and April 30, 2021.

For analysis, patients aged ≥65 years, admitted to the internal medicine ward for 24 h or more and who were not receiving end-of-life palliative care or whose reason for admission was SARS-CoV-2 infection were included.

Those drugs listed in Table 1 ("Potentially Inappropriate Medication Use in Older Adults") of the 2019 AGS Beers criteria were considered PIM. Such table was adapted according to the medications approved for use in Brazil as per data available from Brazil's National Health Surveillance Agency (ANVISA, the Portuguese acronym for "Agência Nacional de Vigilância Sanitária") website on October 28, 2021[16] (Table 2).

An electronic form was created for receiving data taken from the patients' medical records (Soul MV System, version

Table 1. Potentially inappropriate medicines identified in the sample.

Potentially inappropriate medication	Female patients and age		Male patients and age		Total (142)	Percentage of use
	Elderly (65)	Long-lived (20)	Elderly (43)	Long-lived (14)		
Regular human insulin AR	54	18	36	13	121	85.2
Omeprazole	48	15	32	10	105	73.9
Metoclopramide AR	41	12	26	8	87	61.3
Dimenhydrinate AR	4	2	3	1	10	7.0
Promethazine	3	–	2	1	6	4.2
Amiodarone	1	1	2	1	5	3.5
Diazepam AR	2	–	1	1	4	2.8
Dimenhydrinate	3	–	1	–	4	2.8
Phenobarbital	1	2	–	–	3	2.1
Amitriptyline	1	–	1	–	2	1.4
Clonazepam	1	–	1	–	2	1.4
Clonazepam AR	–	–	2	–	2	1.4
Scopolamine	1	–	1	–	2	1.4
Scopolamine AR	2	–	–	–	2	1.4
Hydroxyzine	2	–	–	–	2	1.4
Metoclopramide	2	–	–	–	2	1.4
Propantheline	–	–	2	–	2	1.4
Diazepam	–	–	1	–	1	0.7
Doxazosin	–	–	–	1	1	0.7
Methyldopa	–	1	–	–	1	0.7
Nifedipine	1	–	–	–	1	0.7

AR: as required.

Table 2. Medications licensed for use in Brazil listed in Table 2 of the 2019 American Geriatrics Society Beers criteria.

<table>
<tr><th colspan="5">Therapeutic class</th></tr>
<tr>
<td>Central alpha-agonists
Clonidine (for first-line treatment of hypertension)
Guanabenz
Guanfacine
Methyldopa
Reserpine (>0.1 mg/day)</td>
<td>Antidepressants
Amitriptyline
Amoxapine
Clomipramine
Desipramine
Doxepin (>6 mg/day)
Imipramine
Nortriptyline
Paroxetine
Protriptyline
Trimipramine</td>
<td>First-generation antihistamines
Brompheniramine
Carbinoxamine
Chlorpheniramine
Clemastine
Cyproheptadine
Dexbrompheniramine
Dexchlorpheniramine
Dimenhydrinate
Diphenhydramine (oral)
Doxylamine
Hydroxyzine
Meclizine
Pyrilamine or Mepyramine
Promethazine
Triprolidine</td>
<td>Antiparkinsonian drugs
Benzatropine (oral)
Trihexyphenidyl</td>
<td>Barbiturates
Amobarbital
Butabarbital
Butalbital
Mephobarbital
Pentobarbital
Phenobarbital
Secobarbital</td>
</tr>
<tr>
<td>Genitourinary drugs
Desmopressin</td>
<td>Hormones
Androgens
Methyltestosterone
Testosterone
Other hormones
Thyroid extract
Estrogen with or without progesterone
Growth hormone
Megestrol</td>
<td>Other anxiolytics
Meprobamate</td>
<td>Peripheral alpha-1 blockers for hypertension treatment
Doxazosin
Prazosin
Terazosin</td>
<td>Antispasmodics
Atropine (excludes ophthalmic use)
Belladonna alkaloids
Chlordiazepoxide-clidinium
Dicyclomine
Homatropine (excludes ophthalmic use)
Hyoscyamine
Methscopolamine
Propantheline
Scopolamine</td>
</tr>
<tr>
<td>Nonsteroidal anti-inflammatory drugs
Acetylsalicylic acid (>325 mg/day)
Mefenamic acid
Ketoprofen
Ketorolac (including parenteral)
Diclofenac
Diflunisal
Etodolac
Fenoprofen
Ibuprofen
Indomethacin
Meclofenamate
Meloxicam
Nabumetone
Naproxen
Piroxicam
Sulindac
Tolmetin</td>
<td>Antipsychotics
First generation (conventional) and second generation (atypical)</td>
<td>Benzodiazepines
Short or intermediate action:
Alprazolam
Estazolam
Lorazepam
Oxazepam
Temazepam
Triazolam
Prolonged action:
Chlordiazepoxide (alone or in association with amitriptyline or clidinium)
Clonazepam
Clorazepate
Diazepam
Flurazepam
Quazepam</td>
<td>Nonbenzodiazepine hypnotics (Z-drugs)
Eszopiclone
Zaleplon
Zolpidem</td>
<td>Muscle relaxants
Carisoprodol
Chlorzoxazone
Cyclobenzaprine
Methocarbamol
Orphenadrine</td>
</tr>
<tr>
<td rowspan="2">Other cardiovascular action drugs
Amiodarone Disopyramide
Dronedarone
Digoxin (as first-line treatment for atrial fibrillation or heart failure)
Nifedipine</td>
<td>Antibiotics
Nitrofurantoin</td>
<td>Antithrombotic drugs
Dipyridamole (oral, short-term)</td>
<td rowspan="2">Gastrointestinal tract
Metoclopramide
Mineral oil (oral)
Proton-pump inhibitor</td>
<td rowspan="2">Hypoglycemic drugs
Sulfonylureas of prolonged action
Chlorpropamide
Glimepiride Glyburide or Glibenclamide
Short or fast-acting insulins (in a scheme according to capillary glycemia)</td>
</tr>
<tr>
<td>Opioids
Meperidine</td>
<td>Vasodilators of dubious efficacy
Ergoloid mesylates
Isoxsuprine</td>
</tr>
</table>

SMA-PEP.2019.006.LTS®). The following variables were collected: sex, age, ethnicity, comorbidities, prescription (at day 2 of hospital stay), and length of stay.

Elderly patients were those aged 65 years or older, and those aged 80 years or older were termed long-lived.

In addition, the Charlson index was also calculated[17] from the collected data.

This work is part of project no. 22314819.4.0000.5479 approved by the Research Ethics Committee (CEP) of the institution where it was carried out.

Statistical analysis

Statistical analysis of the study population was based on the presence or absence of PIM in the patient's prescription.

The Charlson index was also compared between groups of male and female patients, as well as between elderly and long-lived patients, for whom Pearson's χ^2 test was used with a significance level (alpha value) of 0.05.

RESULTS

A total of 142 patients (85 females and 57 males) with a mean age of 74.5±7.3 years (65–99 years) were analyzed. Of them, 108 (76.1%) were elderly and 34 (23.9%) were long-lived. The mean length of stay in the sample was 25.3±28.7 days (from 2 to 235 days), with 102 of the 142 analyzed patients having stayed at the hospital for up to 29 days.

Sixteen drugs considered PIM were found in the patients' prescriptions (Table 1).

The elderly had a mean of 2.57±0.94 PIMs prescribed, whereas the long-lived patients had 2.56±0.89, with at least one PIM having been prescribed for 141 (99.3%) patients.

The Charlson index was calculated according to sex (female or male) and age (elderly or long-lived) of the sample (Table 3).

Table 3. Study population's Charlson index.

Charlson index	Sex (F/M) p=0.047*	Age (E/L) p=0.049*	Total
Absence of comorbidity	33 (39)/18 (32)	37 (35)/14 (41)	51 (36)
Low comorbidity	32 (38)/14 (25)	31 (29)/15 (44)	46 (33)
High comorbidity	20(23)/24 (43)	39 (36)/5 (15)	44 (31)

F: female; M: male; E: elderly; L: long-lived.

DISCUSSION

With aging, there occur losses to the functional reserve of multiple organs, which affects drug metabolism. PIMs for the elderly, therefore, are those whose use represents a greater potential risk than benefit for this population.

This, along with the global trend of an increasing number of elderly people, makes not only the identification but also the avoidance of PIM use in health institutions essential for preventing potentially negative outcomes, such as predictable adverse drug reactions, prolonged length of stay, and disabilities.

Literature data on PIM use, especially in developing countries, remain scarce, as does the analysis of the prevalence of these drugs in an in-hospital environment, since a significant portion of the studies focuses on institutionalized elderly patients or in outpatient care. This study, therefore, aims to address this gap.

Our sample showed significant percentages of PIM prescriptions, with 99.3% of them making use of at least one PIM. The prescription of some PIMs "as required" was also noted, which does not minimize their harmful risk, since in an in-hospital environment their use can become daily rather than episodic.

The drugs that were prescribed in our series are considered inappropriate due to the exacerbation of their mechanism of action in the elderly population. Thus, changes that are inherent to aging, such as reduced activation of enzymatic systems, lower concentration of plasma proteins, impaired renal function, and changes in body composition (in which case, the liposubstitution process alters the expected distribution of lipophilic drugs), contribute to greater bioavailability of such drugs and may result in actual doses exceeding the therapeutic dose that would be desired, therefore causing toxic effects[15,18].

Among the drugs identified, regular human insulin, omeprazole, and metoclopramide stand out as those most prescribed ones. The first is considered inappropriate when it is not used concomitantly with long-acting insulin, due to the increased risk of hypoglycemia and inappropriate management of hyperglycemia, which contributes to its long-term consequences[15].

It is noteworthy, however, that the health care service where our study was carried out began to implement a protocol for insulin prescription after our data collection had finished. Such a protocol eliminates the status of this drug as PIM, which can result in a significant reduction in its prescription.

In relation to metoclopramide, its contraindication is due to the risk of extrapyramidal symptoms. Dystonia and akathisia are symptoms that can occur following administration of a single dose of this drug, whereas conditions such as Tardive dyskinesia and secondary Parkinsonism tend to occur with sustained use[15].

The use of omeprazole, in turn, was associated with an increased risk of bone loss, falls and fractures, infection by *Clostridium difficile*, dementia, vitamin B12 deficiency, and kidney disease. It is worth mentioning that such effects have been reported when it was used in a chronic manner (at least one daily dose for 8 weeks)[19], with its use being more relevant in the in-hospital environment for prolonged stays.

The Charlson index is a method for categorizing patients' comorbidities that can be used as a prognostic tool for hospital mortality. The fact that in our study population, female and long-lived patients had better rates coincides with the tendency of these populations to have healthier lifestyle habits, in addition to seeking primary health care services more often, which allows for the prevention of diseases or screening thereof with an early treatment and hence better progression.

CONCLUSIONS

This work has contributed to identifying the indiscriminate prescription of PIMs for the elderly population in tertiary health care centers. It thus serves as an alert to health care professionals about the importance of recognizing such indiscriminate use, assisting clinical practice, and optimizing patient care.

AUTHORS' CONTRIBUTIONS

RMAFO: Data curation, Formal Analysis, Writing – original draft. **MLG:** Conceptualization, Writing – review & editing. **RFR:** Conceptualization, Methodology, Writing – review & editing.

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ORIGINAL ARTICLE

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Predictors of left ventricular ejection function decline in young patients with ST-segment elevation myocardial infarction

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SUMMARY

OBJECTIVE: A decrease in the left ventricular ejection fraction (≤40%) in the setting of ST-segment elevation myocardial infarction is a significant predictor of mortality in the young ST-segment elevation myocardial infarction population. In this study, we aimed to investigate the predictors of left ventricular ejection fraction reduction and evaluate the long-term mortality rates in young ST-segment elevation myocardial infarction patients with or without decreased left ventricular ejection fraction.

METHODS: We enrolled retrospectively 411 consecutive ST-segment elevation myocardial infarction patients aged 45 years or below who underwent primary percutaneous coronary intervention. Young ST-segment elevation myocardial infarction patients were divided into two groups according to their left ventricular ejection fraction (≤40%, n=72 and >40%, n=339), which were compared with each other.

RESULTS: Statin use, white blood cell count, C-reactive protein, peak creatine kinase-MB, prolonged ischemia time, left anterior descending artery-related infarction, proximally/ostial located lesion, and no-reflow were independently associated with low left ventricular ejection fraction. Additionally, long-term mortality was considerably higher in the left ventricular ejection fraction ≤40% group than those in the left ventricular ejection fraction>40% group (18.1% versus 2.4%; p<0.001).

CONCLUSIONS: In young ST-segment elevation myocardial infarction patients, lesion properties (left anterior descending lesion, proximally located lesion), no-reflow, and prolonged ischemia time appeared to be important determinants for the left ventricular ejection fraction decline, rather than coronary disease severity or demographic and hematological parameters. Statin use may be preventive in the development of left ventricular ejection fraction decline in young ST-segment elevation myocardial infarction patients.

KEYWORDS: STEMI. Mortality. Adult. Percutaneous coronary intervention.

INTRODUCTION

The incidence of ST-segment elevation myocardial infarction (STEMI) is 0.05–0.15% annually, and a significant number of STEMIs (5.5–11.6%) have been found at a young age (≤45 years)[1-3]. Although in-hospital and long-term mortality rates are better in younger patients with myocardial infarction than in the older population, compared with the general male population, the risk of mortality is 2–4 times higher in men and even higher in women[4,5]. To date, for different age groups or general STEMI patients, many parameters related to mortality have been introduced, including Killip class, advanced age, delay in treatment, coronary disease severity, renal failure, left ventricular ejection fraction (LVEF), post-percutaneous coronary intervention (PCI), thrombolysis in myocardial infarction (TIMI) flow, and noncompliance with pharmacological recommendations[1,3,6]. In young patients, LVEF decrease (≤40%) in the course of STEMI is a strong predictor of mortality, consistent with the general STEMI population[6]. Aside from being a significant predictor of mortality, the reduced LVEF is also associated with reduced functional capacity and quality of life and with increased rehospitalization and the economic burden in surviving patients after myocardial infarction[7].

In young STEMI patients, the precise predictors of decreased LVEF, which is associated with poor outcomes, have not yet been discovered. In this study, we aimed to

1) investigate the predictors of LVEF reduction and;
2) evaluate the long-term mortality rates in young STEMI patients with LVEF>40% and LVEF≤40% who were treated with primary PCI (pPCI).

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METHODS

Study population

This study was performed in accordance with the Principles of the Declaration of Helsinki and was approved by the Institutional Ethics Committee. We conducted this study by retrospectively enrolling consecutive 435 patients aged 45 years or below with STEMI who underwent pPCI between January 2012 and January 2017. Of these, 24 were excluded from the study because of previously known myocardial infarction and/or heart failure (n=16) and missing clinical and/or long-term follow-up data from hospital files (n=8). Thus, the final study consisted of 411 patients. Telephone interviews, hospital records, and the death registry database were the sources of long-term follow-up data. STEMI was defined according to the current guidelines[1].

Data collection

Patients' medical history and data on baseline clinical and demographic characteristics were obtained from hospital records and patient files. These records indicated that blood biochemical parameters and a complete blood count had been obtained for all patients upon admission to the hospital. Blood samples were retested every 6 h for creatine kinase-myocardial band (CK-MB) and troponin T until peak levels were detected. LVEF obtained before discharge, which was assessed using a modified version of Simpson's method, was considered in the study.

The digital angiograms (Dicom-viewer; MedCom GmbH, Darmstadt, Germany) of all patients who were treated with pPCI by experienced interventional cardiologists were analyzed quantitatively in terms of lesion and intervention characteristics. The coronary blood flow patterns before and after pPCI were evaluated based on TIMI flow grade, and epicardial no-reflow was defined as a TIMI flow grade <3 in the target vessel lesion. The thrombus burden was assessed according to the TIMI thrombus grading scale, as defined previously[8]. The patients' SYNergy between Percutaneous Coronary Intervention with TAXus and cardiac surgery (SYNTAX) score was calculated using the online SYNTAX score calculator (www.syntaxscore.org) to indicate the severity of coronary artery disease.

Statistical analysis

SPSS version 22.0 (SPSS, Inc., Chicago, IL, USA) was used for the statistical analysis. The Kolmogorov-Smirnov test was used to analyze the normality of the data. Continuous variables with normal distribution were expressed as mean±standard deviation and were compared using the independent t-test. Non-normal data were expressed as median (0.25–0.75 percentiles) values and compared using the Mann-Whitney U test. Fisher's exact test or χ^2 test was used to compare the categorical variables which were expressed as percentages. Multivariate logistic regression analyses were performed to identify the independent predictors of reduced LVEF, using the variables that showed a marginal association in the univariate analysis. Power analysis was performed with G*Power version 3.1.9.4 and the power values obtained in the post-hoc power analysis of the parameters found as predictors in the logistic regression analysis were between 0.684 and 0.988. The Kaplan-Meier survival curve analysis was used to demonstrate the event-free survival curves of the patients with LVEF ≤40% or >40%, and the log-rank test was used for comparison. A p-value of <0.05 indicated statistical significance.

RESULTS

The study population consisted of 411 young STEMI patients (mean age: 40±4 years; 8.5% female) who underwent pPCI. The average LVEF of the patients was 47.28±8.76. The patients were divided into two groups according to their LVEF values: the high (>40%) LVEF group (n=339, mean LVEF: 50.30±6.13) and the reduced (≤40%) LVEF group (n=72, mean LVEF: 33.07±3.92). The baseline characteristics of all the patients and those of the low and high LVEF groups are shown in Table 1. In patients in the low LVEF group, diabetes mellitus and dyslipidemia were more common. Patients in the low LVEF group had a higher prevalence of Killip class >1 (on admission), higher heart rate, and higher values of white blood cells (WBC), neutrophils, neutrophil-to-lymphocyte ratio (NLR), blood glucose, peak CK-MB, and C-reactive protein (CRP) than those in the high LVEF group. Furthermore, patients in the low LVEF group had lower levels of hemoglobin and estimated glomerular filtration rate than those in the high LVEF group. In comparing the properties of angiography and ischemia, patients in the low LVEF group had a longer total ischemia time and a higher SYNTAX score than those in the high LVEF group. Infarct-related artery (IRA) of the left anterior descending (LAD), proximal/ostial localization of the culprit lesion, no-reflow phenomenon, and high-grade thrombus burden were more frequent in the low LVEF group. The rate of long-term mortality was found to be considerably higher in the low LVEF group than in the high LVEF group (Table 1).

Table 1. Characteristics of all patients and patient groups with low and high Left ventricular ejection fraction.

	Left ventricular ejection fraction (LVEF)						
	All patients		LVEF>40 (n=339)		LVEF≤40(n=72)		p-value
Age (years)	40	±4	40	±4	41	±4	0.403
Female gender n (%)	35.0	(8.50)	33.0	(9.70)	2.0	(2.80)	0.055
Diabetes mellitus n (%)	57.0	(13.90)	40.0	(11.80)	17.0	(23.60)	**0.009**
Hypertension n (%)	67.0	(16.30)	51.0	(15.00)	16.0	(22.20)	0.135
Dyslipidemia n (%)	213.0	(51.80)	118.0	(34.80)	40.0	(55.60)	**0.001**
Family history of CAD n (%)	132.0	(32.10)	113.0	(33.30)	19.0	(26.40)	0.252
Smoking n (%)	319.0	(77.60)	264.0	(77.90)	55.0	(76.40)	0.784
Medications							
Acetylsalicylic acid n (%)	4.0	(1.00)	2.0	(0.60)	2.0	(2.80)	0.086
β-Blocker n (%)	28.0	(6.80)	22.0	(6.50)	6.0	(8.30)	0.573
ACEI/ARB n (%)	35.0	(8.50)	28.0	(8.30)	7.0	(9.70)	0.687
Statin n (%)	83.0	(20.20)	78.0	(23.00)	5.0	(6.90)	**0.002**
Insulin n (%)	7.0	(1.70)	5.0	(1.50)	2.0	(2.80)	0.438
Killip class >1 on admission n (%)	60.0	(14.60)	35.0	(10.30)	25.0	(34.70)	**<0.001**
Arrest on admission n (%)	13.0	(3.20)	11.0	(3.20)	2.0	(2.80)	0.837
Systolic blood pressure (mm Hg)	124	±24	125	±21	118	±35	0.196
Heart rate (bpm)	78	±15	76	±13	88	±17	**<0.001**
Hemoglobin (g/dL)	14.5	±1.7	14.6	±1.4	13.9	±2.4	**<0.001**
White blood cell count (×10^9/L)	13.61	±3.85	12.85	±3.13	17.14	±4.83	**<0.001**
Platelet count (×10^9/L)	271	±74	271	±73	270	±79	0.797
Neutrophil count (×10^9/L)	10.37	±3.87	9.59	±3.21	14.05	±4.58	**<0.001**
Lymphocyte count (×10^9/L)	2.00	(1.50–3.00)	2.10	(1.50–3.00)	1.65	(1.40–2.60)	0.074
Neutrophil-to-lymphocyte ratio	4.87	(2.79–7.92)	4.38	(2.60–7.06)	7.19	(4.63–12.26)	**<0.001**
Blood glucose on admission (mg/dL)	118	(102–144)	115	(101–142)	127	(109–179)	**0.003**
C-reactive protein (mg/dL)	8.76	(4.52–16.50)	7.74	(4.32–13.20)	24.50	(15.40–45.00)	**<0.001**
Serum albumin (g/dL)	3.95	±0.49	3.93	±0.46	4.05	±0.60	0.109
Estimated glomerular filtration rate (mL/min)	102.01	±20.44	103.20	±20.09	96.38	±21.27	**0.049**
Peak creatine kinase MB (ng/mL)	171	99-308	143	87-235	478	373-678	**<0.001**
LVEF (%)	47.28	±8.76	50.30	±6.13	33.07	±3.92	**<0.001**
Total ischemia time (min)	166	110–254	145	95–217	270	172–430	**<0.001**
LAD as the infarct-related artery n (%)	254	(61.80)	184	(54.30)	70	(97.20)	**<0.001**
Proximal/ostial lesion for IRA n (%)	236	(57.40)	175	(51.60)	61	(84.70)	**<0.001**
High-grade thrombus burden n (%)	259	(63.00)	193	(56.90)	66	(91.70)	**<0.001**
No-reflow n (%)	35	(8.50)	14	(4.10)	21	(29.20)	**<0.001**
Left main coronary artery n (%)	5	(1.20)	5	(1.50)	0	(0.00)	-
Three vessels disease n (%)	25	(6.10)	18	(5.30)	7	(9.70)	0.155
Presence of chronic total occlusion n (%)	28	(6.80)	22	(6.50)	6	(8.30)	0.573
Basal syntax score	16.41	±4.04	15.89	±4.10	18.85	±2.68	**<0.001**
Long-term mortality n (%)	21	(5.1)	8	(2.4)	13	(18.1)	**<0.001**
Follow-up time (month)	38	±13	39	±11	31	±19	

ACEI: angiotensin-converting enzyme inhibitors; ARB: angiotensin-receptor blocker; LVEF: left ventricular ejection fraction; LAD: left anterior descending; IRA: infarct-related artery. Bold indicates significant p-value.

Multivariate regression analysis was performed to determine the independent predictors of reduced LVEF, using the parameters found to be associated with reduced LVEF in the univariate analysis. Statin use, WBC, CRP, peak CK-MB, total ischemia time, LAD as the IRA, proximal/ostial lesion for IRA, and no-reflow were found to be independently associated with low LVEF (Table 2).

During an average follow-up of 38±13 months, 21 (5.1%) deaths from all causes were reported. The rate of long-term mortality was significantly higher among patients in the low LVEF group than among those in the high LVEF group (n=13, 18.1% versus n=8, 2.4%; p<0.001). The Kaplan-Meier survival curve of long-term mortality is shown in Figure 1.

DISCUSSION

In this study, we evaluated the predictors of reduced LVEF in patients with STEMI aged ≤45 years. The demographic features were not determined as predictors of decreased LVEF development, whereas statin use from the pharmacological history was found to be protective in the occurrence of decreased LVEF. While WBC and CRP were independent predictors of reduced LVEF, NLR, as an inflammatory parameter, was not a predictor of reduced LVEF. The most considerable findings of this study were that lesion localization, procedure characteristics (i.e., IRA, proximal/ostial lesion, and no-reflow), and prolonged ischemia time were the main causes of reduced LVEF.

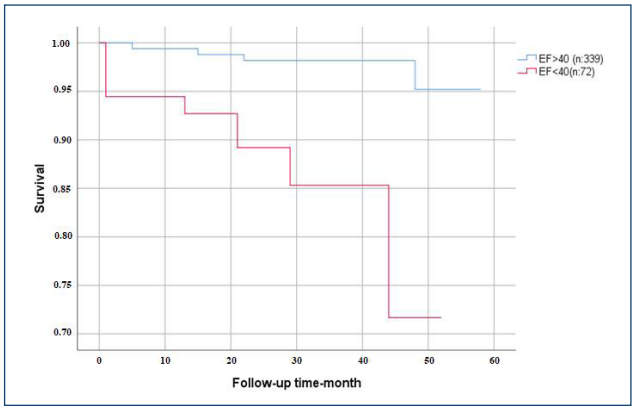

Figure 1. Kaplan-Meier long-term survival curve of patients with low and high left ventricular ejection fraction.

Table 2. Univariate and multivariate logistic regression analysis of characteristics for prediction of reduced LVEF (LVEF≤40).

Variable	Univariate analysis of reduced LVEF			Multivariate analysis of reduced LVEF		
	Odds ratio	95%CI	p-value	Odds ratio	95%CI	p-value
Female gender	0.265	0.062–1.130	0.073	–	–	–
Diabetes mellitus	2.310	1.223–4.365	**0.010**	–	–	–
Dyslipidemia	1.157	1.034–1.434	**0.040**	–	–	–
Statin use	0.250	0.097–0.641	**0.004**	0.011	0.001–0.117	**<0.001**
Hemoglobin (g/dL)	0.773	0.668–0.895	**<0.001**	–	–	**–**
White blood cell count (×10⁹/L)	1.338	1.236–1.449	**<0.001**	1.947	1.156–3.278	**0.012**
Neutrophil count (×10⁹/L)	1.360	1.252–1.477	**<0.001**	–	–	**–**
Lymphocyte count (×10⁹/L)	0.880	0.709–1.091	0.244	–	–	**–**
Neutrophil-to-lymphocyte ratio	1.163	1.098–1.233	**<0.001**	–	–	**–**
Basal blood glucose level (mg/dL)	1.003	1.000–1.006	0.340	–	–	**–**
C-reactive protein (mg/dL)	1.120	1.088–1.153	**<0.001**	1.123	1.054–1.197	**<0.001**
Peak creatine kinase MB (ng/mL)	1.012	1.009–1.015	**<0.001**	1.018	1.011–1.025	**<0.001**
Total ischemia time (min)	1.008	1.006–1.010	**<0.001**	1.018	1.010–1.027	**<0.001**
LAD as IRA	29.484	7.114–122.187	**<0.001**	218.725	13.049–3666.318	**<0.001**
Proximal/ostial lesion for IRA	5.197	2.642–10.222	**<0.001**	1.033	1.005–1.245	**<0.001**
No-reflow	8.321	3.511–19.722	**<0.001**	15.311	2.271–103.252	**0.005**
High-grade thrombus burden (Grade 4/5)	9.559	4.570–19.192	**<0.001**	–	–	–
Syntax score	1.200	1.120–1.286	**<0.001**	–	–	–

LVEF: left ventricular ejection fraction; CI: confidence interval; LAD: left anterior descending; IRA: infarct-related artery. Bold indicates significant p-value.

The fact that LVEF is closely related to death and poor quality of life and that data about reduced LVEF predictors in young STEMIs are lacking has prompted us to investigate the predictors of LVEF decline in young STEMI patients. In our study, the demographic characteristics of the patients, including diabetes and hyperlipidemia, were not predictors of reduced LVEF. The reason that diabetes is not related to LVEF may be that many years are required to develop microvascular dysfunction and diabetic cardiomyopathy[9]. In parallel with previous randomized studies showing that statin use could reduce the risk of developing heart failure, in our study, the use of statin was a predictor of preventing the development of heart failure in young STEMI patients[10,11].

Ischemic injury induces an inflammatory response, the intensity of which is an important predictor of ventricular remodeling. The CRP levels in STEMI patients have been shown to be closely associated with infarct size, reduced LVEF, and left ventricular volumes, aside from mortality[12]. Similarly, NLR, as a recently identified inflammatory parameter, has been found to be a predictor of LVEF decline and mortality for unselected STEMI patients[13]. In our study, CRP was an independent predictor of reduced LVEF in young STEMI patients, which is consistent with the general STEMI cohort, but NLR was not.

Studies investigating the relationship between the infarct location/size and prognosis have shown that patients with a large infarct size (mostly confirmed by a high peak enzyme level) had a poor in-hospital and long-term prognosis and a reduced LVEF[14]. Similarly, no-reflow has been found to be a strong determinant of infarct size and LVEF decrease[15]. In our study, proximally located and LAD-related STEMIs and no-reflow were found to be predictors of reduced LVEF in young STEMI patients, consistently with the aforementioned studies. Moreover, CK-MB was higher in patients with lower LVEF and is a predictor of LVEF decline.

Delay in reperfusion therapy has been shown to be associated with both mortality and LVEF reduction[16]. In patients with delayed reperfusion, the LVEF decline is mostly attributed to increased infarct size. In the present study, prolonged total ischemia time was found to be an independent predictor of reduced LVEF in young STEMI patients, similar to the general STEMI population.

Previous studies evaluating mortality rates of young STEMI patients reported a mortality rate of 3–4%[3,17]. We also found the long-term mortality rate (for 38±13 months) of patients with STEMI aged ≤45 years in the present study was 5.1%. LVEF was reduced to an average of 47.28%, and the rate of patients with reduced LVEF (≤40%) was 17.5% in the present study. This rate was consistent with the study investigating reduced LVEF following STEMI in a general STEMI cohort[18]. In the present study, the rate of long-term mortality was considerably higher in the low LVEF group than in the high LVEF group (18.1 versus 2.4%) in young STEMI patients. This finding was consistent with a recent study in young STEMI women, which reported that every 5% increase in LVEF at discharge reduced the mortality rate by 60%[6].

The possible clinical implication of our study is that revealing the factors associated with LVEF decline more precisely in young STEMI patients may substantially not only contribute to the development of new strategies in STEMI treatment and a reduction of the LVEF decline and its associated mortality rates for this specific patient group but also allow us to identify patients who are at higher risk of developing a reduced LVEF and, therefore, require closer clinical follow-up.

This study has some limitations. Although we determined the adequacy of our sample size by comparing it with similar studies in the literature and performing power analysis, our results should be validated in larger clinical trials. Although cardiac magnetic resonance imaging (CMRI) is the gold standard for assessing left ventricular function, CMRI could not be performed owing to the retrospective nature of the study and the high cost and limited availability of CMRI. LVEF measured before discharge was used in our study and no repeated measurements during the follow-up period were taken into account, as they were beyond the scope of the study. This study had a retrospective design and was based on a registry analysis. As the patients included in the study were young and had experienced their first myocardial infarction, the current reduced LVEF was attributed to their recent STEMI. That is, although there were no data, the presence of heart failure extending before STEMI could not be excluded.

CONCLUSIONS

In young STEMI patients, lesion localization (LAD lesion, proximally located lesion), no-reflow, and prolonged ischemia time seem to be important determinants of the LVEF decline, rather than coronary disease severity or demographic and hematological parameters. Moreover, statins should be used in dyslipidemic young patients to avoid procedural transactions that could cause no-reflow.

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This study protocol was approved by the Ethics Committee of the Kafkas University Medical Faculty. This study was conducted under the principles of the Declaration of Helsinki.

Written informed consent was waived owing to the retrospective nature of the study.

AUTHORS' CONTRIBUTIONS

IY: Conceptualization, Data curation, Formal Analysis, Methodology, Writing – original draft, Writing – review & editing. **IR:** Conceptualization, Data curation, Formal Analysis, Writing – original draft, Writing – review & editing. **YK:** Conceptualization, Data curation, Formal Analysis, Writing – review & editing. **MK:** Data curation, Methodology, Writing – review & editing. **IA:** Data curation, Methodology, Writing – review & editing. **MSG:** Data curation, Methodology, Writing – review & editing.

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ORIGINAL ARTICLE

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Characteristics of patients receiving nutrition care and its associations with prognosis in a tertiary hospital

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SUMMARY

OBJECTIVE: The aim of this study was to describe the medical nutritional therapy (MNT) of adult non-critically ill hospitalization patients.

METHODS: In a retrospective study, adults hospitalized for more than 48 h in non-intensive care unit medical and surgical areas that were classified as being at nutritional risk were included. Malnutrition was defined according to Global Leadership Initiative on Malnutrition (GLIM) criteria.

RESULTS: A total of 255 patients, aged 54.13±18.4 years, who were at risk of malnutrition were included in this study. Of these, 50% were males. Notably, 52.5% received oral nutrition supplementation (ONS), 23.5% enteral nutrition (EN), 15% parenteral nutrition (PN), and 9% received enteral and parenteral nutrition (EPN). Patients with EPN presented the highest frequency of malnutrition (52%), and therefore they received more than 100% of energy and protein requirements. The median length of stay was 25 days. Among patients with nutritional risk receiving EPN, no deaths occurred. Patients, identified at nutritional risk, but without malnutrition according to GLIM, and receiving ONS had significantly lower mortality than patients receiving other MNT.

CONCLUSIONS: Oral nutrition supplementation was the more frequent MNT prescribed. The frequency of malnutrition and percentage of prescribed energy and protein were higher in patients receiving PN and EPN compared with those receiving ONS.

KEYWORDS: Nutritional therapy. Malnutrition. Malnutrition. Hospitalization. Adults.

INTRODUCTION

Disease-related malnutrition in hospitalized patients is a major public health problem, with a reported prevalence from 40 to 60% at admission in Latin American[1] and Mexico. In a previous study, we found 44.2% of patients with severe malnutrition[2]. This could be due to insufficient nutrient intake, with anorexia as a physiological factor, impaired absorption or loss of nutrients due to illness or trauma, or increased metabolic demands during illness[1,3].

Disease-related malnutrition is associated with muscle wasting poor wound healing, impaired immune function, longer hospital stay, and higher morbidity and mortality[3-5]. Given these consequences, current clinical practice guidelines recommend to consider initiating medical nutritional therapy (MNT) during the hospital stay of malnourished medical inpatients or those at risk of malnutrition in order to increase the uptake of essential nutrients and improve clinical outcomes[6,7].

However, current knowledge and guidelines of MNT for polymorbid hospitalized medical patients remain unclear and have been derived from clinical trials or systematic reviews based on critical care or surgical populations[5,8]. Patients with multiple comorbidities often have varying degrees of chronic malnutrition exacerbated by acute or chronic medical illness, which presents challenges to the nutritional and metabolic milieu. In addition, even negative outcomes of nutrition interventions have been reported[6,9].

The aim of this study was to describe the MNT of adult non-critically ill hospitalization patients and its association with in-hospital mortality and intensive care unit (ICU) transfer.

METHODS

An observational, retrospective study was conducted during the period of 2016–2020 at Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán (INCMNSZ). Adults hospitalized for more than 48 h in non-ICU medical and surgical areas that were classified as being at nutritional risk and managed by the Clinical Nutrition Service were included and those with oral nutrition and uncompleted/partial charts in medical records were excluded.

The protocol for the research project was approved by the ethics committee at the institution.

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Data on characteristics, such as patient sex, age, principal hospital admission diagnosis, date of admission and date of discharge, and MNT prescription, were obtained from electronic medical charts and were captured in an Excel 2013 worksheet. In addition, Charlson Comorbidity Index was obtained, taking into consideration both the number and severity of comorbidities, based on a number of comorbidities that are each assigned an integer weight from 1 to 6, with a weight of 6 representing the most severe morbidity. The summation of the weighted comorbidity scores results in a summary score[10].

Malnutrition risk was evaluated using the Nutritional Risk Screening-2002 (NRS-2002) tool by standardized nutritionists within 24 h after admission according to ESPEN guidelines[11]. Patient with a total score of ≥3 were classified as being at nutritional risk[12].

Information about food intake and weight loss (compared to the usual weight) in last week were collected by interviewing the patients or, in the presence of altered mental status or impaired communication, their relatives/caregivers. Reduction of food intake was estimated by assessment of food consumed in the week before admission compared with the usual intake. Usual weight was obtained to calculate the percentage of body weight loss before admission. The severity of disease was scored according to the patient's history and reason of acute hospital admission[11].

Weight and height were measured during the medical stay in the hospital. Body mass index (BMI) was calculated by dividing the total body weight (kg) by height (m) squared.

Percentage of food intake of preceding week before admission was evaluated with a Visual Comstock Scale[13].

The diagnosis of malnutrition was made according to the GLIM criteria as follows: etiologic criterion of reduced food intake ≤50% in 1 week and phenotypic criterion with non-volitional weight loss >5% in 6 months or low BMI <20 (if age <70 years) or <22 (if age ≥70 years)[14].

The calorie and protein requirements for MNT prescription were based on a validated "Algoritmo para el Soporte Nutricional Enteral Total" (ASNET) developed in our Service according to systematic literature search and international guidelines[15]. The adequacy of the energy and protein requirements was calculated based on the overall caloric protein received (from hospital admission until discharge) divided by the amount prescribed, represented as a percentage.

Follow-up information was obtained using the medical charts.

Statistical analysis

The differences among subgroups of route of feeding (i.e., oral nutrition supplementation [ONS], enteral nutrition [EN], parenteral nutrition [PN], and enteral and parenteral nutrition [EPN]) were assessed by a chi-square test in categorical variables and by Kruskal-Wallis test or an analysis of variance (ANOVA) in continuous variables, and a post-hoc analysis with a Tukey test was performed for continuous variables with $p<0.05$ in ANOVA simple comparison.

Kaplan-Meier survival curves with log-rank significance tests were also performed to assess survival based on the presence or absence of malnutrition. The data were analyzed using Jamovi version 1.6.15 and the Statistical Package for the Social Sciences version 21.

RESULTS

A total of 686 hospitalized patients during 2016–2020 were classified as being at risk of malnutrition. Of these, 431 were excluded since they received oral nutrition, and 255 were included in the study. In all, 50% were males, aged 54.13±18.4 years, and 52.5% received ONS, 23.5% EN, 15% PN, and 9% EPN during a hospital stay that lasted 3 days or more. The gastrointestinal primary cause of hospital admission was the most frequent in all patients.

Weight and BMI were significantly lower and the frequency of malnutrition was higher in patients receiving EPN compared with those receiving EN. Patients receiving ONS were more likely to have lower NRS scores (Table 1).

The characteristics of MNT prescription are presented in Table 2 and we can see that the adequacy of protein and energy requirements was higher in the PN and EPN groups and lower in ONS group.

The proportion of patients with EN who received gastric feeding was 59.2% (11.1% by nasogastric tube; 22.3% received by jejunostomy, and 18.5% received by nasoenteric tubes). Specialized EN formula was prescribed in 23.8%, polymeric formulas in 76.2%, and extra protein modular supplement in 46% by continuous infusion (95%). On average, initial rate was 31.2±22 mL/h and progression rate was 56.9±16.1.

In all patients with PN, this was administered through venous central catheter.

The length of stay was significantly higher in EPN group. Interestingly, no deaths occurred during hospital stay in this group. On the contrary, EN and PN groups presented higher mortality and higher frequency of patients who were transferred to ICU (Table 3).

Figure 1 presents the Kaplan-Meier survival curves of in-hospital mortality based on the presence or absence of malnutrition. Patients without malnutrition with ONS had significantly lower mortality than patients with EN or PN. No

deaths occurred among patients receiving EPN. With respect to patients with malnutrition, patients with ONS presented higher mortality than patients with MNT, but these differences did not reach statistical significance.

DISCUSSION

In the present study, according to GLIM criteria, not all the patients with a prescription for medical nutritional support were malnourished, ONS and EN were more frequently provided to

Table 1. Demographic, clinical, and nutritional characteristics of the study population based on route of feeding.

Variables	ONS n=134	EN n=60	PN n=38	EPN n=23	p
Age, years	55±20	56.5±18.3	53.2±18.4	48.6±19.1	0.08
Sex, male (%)	66 (49.2)	32 (53.3)	19 (50)	12 (52.2)	0.29
Primary cause of hospital admission, n (%)					
Surgical	11 (8.4)	5 (8.8)	4 (11.5)	2 (8.3)	0.01
Cardiorespiratory	20 (14.9)	12 (20.1)	2 (6.1)	1 (5.6)	
Gastrointestinal	27 (20.4)	7 (11.8)	15 (38.2)	10 (44.4)	
Neurological disease	3 (2.1)	3 (5.8)	1 (0.6)	1 (2.8)	
Cancer	38 (28.4)	7 (11)	10 (26.1)	5 (22.1)	
Renal disease	7 (5.3)	2 (2.8)	1 (2.4)	0 (0)	
Metabolic disease/infection	19 (14.3)	21 (35.4)	5 (12.1)	3 (13.9)	
Others	4 (3.2)	1 (2.2)	1 (2.6)	0 (0)	
Disease severity, n (%)					
Mild	71 (53)	29 (48.3)	16 (42.1)	16 (70)	0.001
Moderate	62 (46.2)	47 (78)	22 (57.9)	5 (20)	
Severe	1 (0.7)	1 (1.7)	(0)	2 (8.7)	
Charlson comorbidity index (score)	2 (2-3)	2 (2-4)	2 (2-3)	2 (2-2)	0.91
Weight, kg	58.4±19	60.2±14	54.6±17.7	42.5±9.5	0.001*
BMI, kg/m^2	21.8±5.5	22.8±4.5	20.8±6	17.1±4.1	0.001*
Malnutrition, n (%)	26 (19.4)	15 (25)	12 (31.6)	12 (52)	0.001
Obesity, n (%)	8 (6)	4 (7)	2 (5)	0	0.81
NRS 2002 score, n (%)					
3 points	34 (25.6)	7 (12.1)	5 (13.3)	4 (16.7)	0.001
4 points	55 (40.9)	15 (24.5)	11 (27.9)	7 (31.9)	
5 points	40 (29.9)	30 (49.6)	18 (48.5)	10(41.7)	
>5 points	5 (3.7)	8 (13.8)	4 (10.3)	2 (9.7)	
% food intake of last week, n (%)					
>75	22 (16.4)	8 (13)	2 (5.3)	5 (22)	0.001
75–50	41 (30.8)	5 (8)	4 (10.5)	0 (0)	
50–25	30 (22.3)	5 (9.3)	10 (26.3)	2 (8.6)	
<25	41 (30.8)	42 (70.7)	13 (57.9)	15 (65.2)	
% weight loss before admission, n (%)					
<4	63 (47)	12 (20)	6 (15.8)	5 (22)	0.001
5–9	30 (22.3)	6 (10)	10 (26.3)	3 (13)	
>10	41 (30.6)	42 (70)	22 (57.9)	12 (66.7)	

Data are presented as mean±standard deviation or number (%). ONS: oral nutritional supplementation, EN: enteral nutrition, PN: parenteral nutrition, EPN: enteral and parenteral nutrition; BMI: Body mass index; NRS: nutritional risk screening. *EN vs. EPN: p<0.05.

patients with MNT, which is similar to that recommended. Oral intake is optimal because it induces a cephalic-phase response that follows the oral ingestion of food[16].

We reported gastrointestinal primary cause of hospital admission was the most frequent; however, the adequacy of the energy and protein requirements in patients with EN was higher in the present study[2].

Table 2. Nutrition practice in-hospital of the study population based on route of feeding.

Variables	ONS n=134	EN n=60	PN n=38	EPN n=23	p
Goal of energy (kcal)	1626±668	1687±286.8	1550±280	1510±373	0.05
Goal of energy (kcal/kg)	27.9±11.4	28±4.8	28.4±5.2	35.5±8.8	0.05
Energy received (kcal)	1226±504	1421±379	1370±371.3	2380±765	0.001*†
Energy received (kcal/kg)	21±9	23.6±6.3	25.1±6.8	56±18	0.001*†
Energy adequacy (%)	75.4±45	84.2±21.2	90.6±24.5	160.5±34.3	0.001*†
Goal of protein (g)	65.8±11.7	73.2±15.9	70.7±21.3	57.4±10.2	0.01†
Goal of protein (g/kg)	1.1±0.2	1.2±0.26	1.3±0.39	1.3±0.24	0.05
Protein received (g)	50±23	60.2±19.7	70.7±20.5	79.2±30	0.02*†
Protein received (g/kg)	0.9±0.4	1.01±0.33	1.3±0.4	1.8±0.7	0.02*†
Protein adequacy (%)	76±4.2	84.8±25.4	102.4±30.9	138.2±50.4	0.001*†

Data are presented as mean±standard deviation. ONS: oral nutritional supplementation, EN: enteral nutrition, PN: parenteral nutrition, EPN: enteral and parenteral nutrition. *ONS vs. EPN: p<0.05. †EN vs. EPN: p<0.05.

Table 3. Clinical outcomes of the study population based on route of feeding.

Variables	ONS n=134	EN n=60	PN n=38	EPN n=23	p
Length of stay (days)	11 (7-19)	12 (7-20)	16 (9-31)	25 (14-43)	0.01*†
Transferred to ICU	3 (2.2)	4 (6.7)	3 (7.9)	1 (4.3)	0.01
In-hospital mortality, %	4 (3.1)	8 (13.3)	8 (20)	0 (0)	0.01

Data are presented as median (p25th–p75th) or n (%). ONS: oral nutrition supplementation, EN: enteral nutrition, PN: parenteral nutrition, EPN: enteral and parenteral nutrition; ICU: intensive care unit. *ONS vs. PN and EPN: p<0.05. †EN vs. PN and EPN: p<0.05.

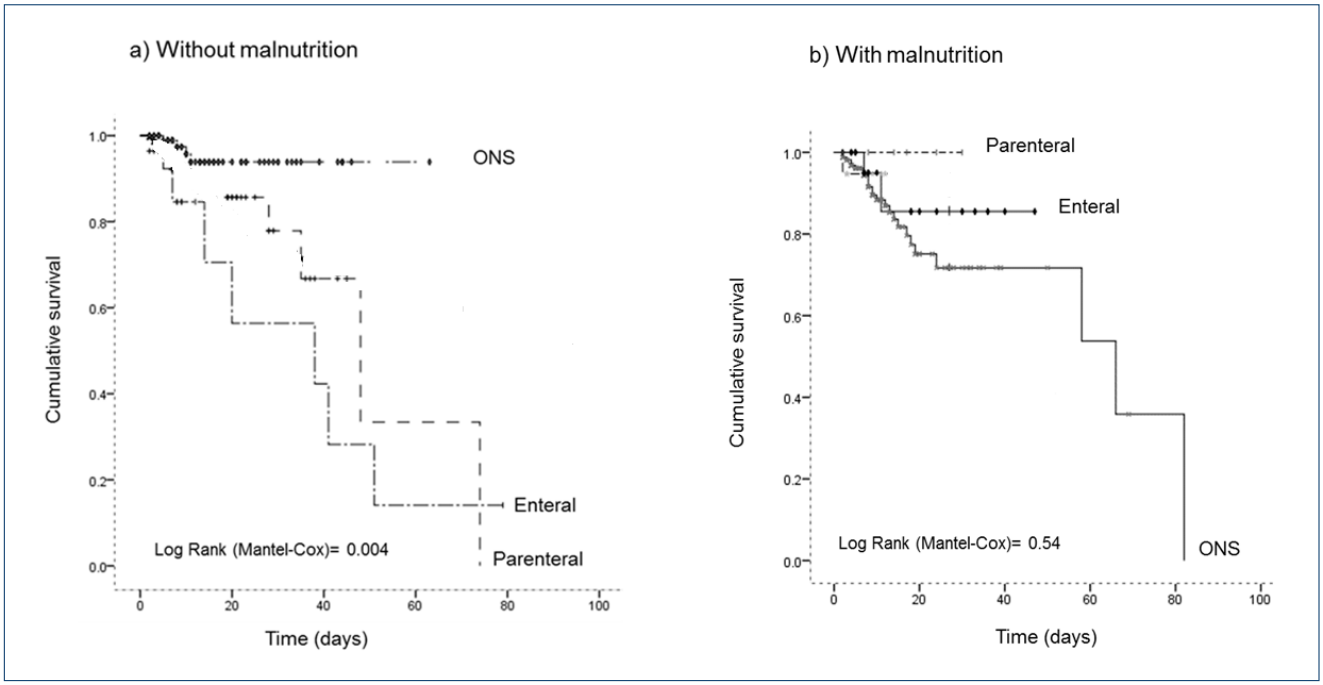

Figure 1. Kaplan-Meier survival for in-hospital mortality based on the (a) presence or (b) absence of malnutrition and route of feeding.

It is interesting that patients with EPN did not present deaths, and this could be due to the fact that this group received a higher percentage (>80%) of prescribed energy and protein and as a result they present longer length of stay; however, this observation can be just an isolated event that should be investigated in future studies to evaluate the potential benefits of the combination of EN and PN, especially in cases where EN alone is not sufficient.

In a recent systematic review and meta-analysis of randomized controlled trials evaluating the effect of supplemental PN versus EN alone on clinical outcomes in critically ill adult patients, the authors found that combined EN and PN improved the protein and energy intake and concluded that when EN fails to meet the energy requirements, PN might be considered at the right time and in the right amount as it helps to increase the energy and protein intake, and PN should be delayed until at least day 4 after the initiation of EN to allow sufficient EN and decrease the amount of PN needed[17].

In a subanalysis between patients with and without malnutrition, we found that patients with EPN presented higher mortality than those with oral nutritional support and no support. This finding is important because it suggests that the screening evaluation of the risk of malnutrition is not sufficient to evaluate malnutrition based on GLIM criteria. Subjects with a risk of malnutrition that is not confirmed with GLIM and who receive MNT can have adverse outcomes. This finding is explained by the fact that the energy and protein adequacy of nutritional therapy was suboptimal, and continuous tube feeding and PN may potentially desynchronize a range of anabolic metabolic processes[9]. Therefore, if not strictly necessary, it is better to not prescribe MNTs because they are invasive and expensive[3].

In patients with malnutrition, we did not find a statistically significant difference in mortality between feedings patients with EPN and those with ONS, as previously mentioned in systematic reviews and meta-analyses of trials addressing nutritional intervention in malnourished medical inpatients[3,5].

The present study investigation has limitations. The results were based on a retrospective study and this study was performed in a single center, which might limit the generalizability of our findings. Also, other prognosis variables such as infections rate and 6 moths follow-up were not evaluated.

CONCLUSIONS

In adults hospitalized for more than 48 h in non-ICU and surgical areas that were classified as being at nutritional risk, ONS was the more frequent MNT prescribed. Patients with EPN present the highest frequency of malnutrition; as a result, they received more than 100% of energy and protein requirements, the median length of stay was 25 days, and no deaths occurred during hospitalization in this group.

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AUTHORS' CONTRIBUTIONS

MTPR: Conceptualization, Data curation, Supervision, Writing – review & editing. **JLVJ:** Methodology, Software, Writing – original draft. **AESZ:** Conceptualization, Project administration, Resources. **LCM:** Formal Analysis, Investigation, Methodology, Writing – review & editing.

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ORIGINAL ARTICLE

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Investigation of serum phoenixin levels in patients with hypertension

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SUMMARY

OBJECTIVE: Hypertension is a major modifiable risk factor for cardiovascular disease and premature death worldwide. Phoenixin is a newly identified neuropeptide with multiple bioactivity. However, there was no published data about phoenixin levels in hypertension. The aim of this study was to evaluate the relationship between phoenixin and hypertension.

METHODS: This study was performed in 36 patients with hypertension and 36 healthy controls. Serum phoenixin-14 and phoenixin-20 levels were determined by Enzyme-Linked ImmunoSorbent Assay method.

RESULTS: Serum phoenixin-14 and phoenixin-20 values were significantly lower in hypertension patients compared with the control group ($p<0.001$). The levels of phoenixin-14 were negatively correlated with weight ($r=-0.376$; $p<0.005$), body mass index ($r=-0.407$; $p<0.001$), systolic blood pressure ($r=-0.586$; $p<0.001$), and diastolic blood pressure ($r=-0.319$; $p<0.01$). There was a negative correlation between serum phoenixin-20 and weight ($r=-0.378$; $p<0.005$), body mass index ($r=-0.383$; $p<0.005$), systolic blood pressure ($r=-0.551$; $p<0.001$), and diastolic blood pressure ($r=-0.306$; $p<0.01$). We used receiver operating characteristic curve analyses to compare the diagnosis value of Phoenixin-14 and Phoenixin-20 levels in hypertensive patients. We found that Phoenixin-14 value is an area under the curve of 0.87 (cutoff value 404.7 ng/L, sensitivity 92%, specificity 72%) and Phoenixin-20 value is an area under the curve of 0.83 (cutoff value 209.9 ng/L, sensitivity 86%, specificity 75%). Phoenixin-14 did nearly show equally compared to phoenixin-20 in predicting hypertension.

CONCLUSION: Serum phoenixin-14 and phoenixin-20 may be related to the pathogenesis of hypertension. Our findings indicated that serum phoenixin-14 and phoenixin-20 may serve as a novel biomarker for the diagnosis of hypertension.

KEYWORDS: Hypertension. Phoenixin-14. Phoenixin-20.

INTRODUCTION

Hypertension (HT) is the most prevalent risk factor for cardiovascular diseases (CVDs). HT affects approximately one-third of the world's adult population and is a major cause of premature death worldwide. Despite technological advances in the diagnosis and treatment of HT, there is still a large population of untreated or inadequately treated HT patients[1]. Understanding the mechanisms for HT development is critical for preventing and treating high blood pressure. The etiopathogenesis of HT is multifactorial and complex. Genetic and lifestyle factors, obesity, insulin resistance (IR), activation of sympathetic nervous system, alteration in sodium homeostasis, renin-angiotensin system changes, changes in vascular smooth muscle structure and reactivity, oxidative stress, and inflammation contribute to the development of HT[1-3].

Phoenixin (PNX) is a recently discovered neuropeptide produced by proteolytic cleavage of a small integral membrane protein 20 (Smim20), with two active isoforms, phoenixin-14 (PNX-14) and phoenixin-20 (PNX-20). PNX is a neuropeptide that is expressed and secreted not only in the central nervous system (CNS) but also in the peripheral tissues such as heart, adipose tissue, and pancreas[4,5]. The original study suggests that PNX is a regulator of the reproductive system. Recent research has shown that PNX is involved in food intake, and lipid and glucose metabolism[6,7]. PNX also exerts cardioprotective, anti-inflammatory, and cell-protective effects[8,9]. However, the role of PNX in HT remains unknown.

Diseases of energetic imbalance such as obesity and diabetes represent major risk factors for CVD such as HT. There is some evidence that PNX may have some effects on the feeding, body mass regulation, and energy homeostasis[6,10]. Oxidative stress and inflammation are considered to play a role in HT development[2,3]. The protective properties of PNX, including antioxidative stress and anti-inflammatory effects, have recently been widely reported[9,11]. PNX-14 has been reported to regulate proliferation and apoptosis of vascular smooth muscle

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cells (VSMCs)[12]. GPR173 agonism by PNX-20 plays a protective role against ox-LDL-induced endothelial dysfunction[13]. Based on these data, this study was designed to evaluate serum concentrations of PNX-14 and PNX-20 in HT patients and healthy controls and the clinical value of these peptides as novel biomarkers for HT.

METHODS

Participants and study design

This study was performed in 36 HT patients and 36 control subjects. The volunteers selected for the patient group are patients with a diagnosis of HT and not having comorbidities. HT patients were selected from those who did not have any additional cardiac complaints such as ischemic or arrhythmic and who were admitted for control for HT. As a rule of exclusion from the research, under the age of 18, having a pregnancy, having comorbidities, having additional complaints, using additional medication other than antihypertensive, and have had COVİD-19 in the past 2 years. All subjects provided written informed consent before participating in the study.

Clinical and biochemical assessment

The patients' body mass index (BMI) was calculated by dividing their weight in kilograms by their height in meters squared. BP was measured using an automatic BP monitor in the sitting position, and measurements were performed three times in the participant's right arm with a 2-min interval, after a rest period of at least 5 min. Samples of venous blood were taken after overnight fasting for at least 10 h. Serum biochemical analyte levels were measured immediately by commercially available kits based on routine methods on the Roche Cobas c501 analyzer (Roche Diagnostics, Mannheim, Germany). Blood samples were centrifuged and then serum samples were stored at -80°C for subsequent analysis. The analyses of serum PNX-14 and PNX-20 levels were performed using an enzyme immunoassay method using commercial kits (BT Lab Bioassay Technology Laboratory Human Elisa Kits, Shanghai Korain Biotech, China) in accordance with the manufacturer's guidelines. Absorbance was measured at 450 nm on an ELx800 Absorbance Microplate Reader (Biotek, Winooski, VT, USA).

Statistical analysis

Statistical analyses were done using SPSS v. 22.0 (SPSS Inc., IL, USA). To compare the ratio of categorical variables, we used the chi-squared test [gender (male/female)]. The normality of the variables was evaluated using the one-sample Kolmogorov-Smirnov test.

Differences in the means of variables were tested using both parametric and nonparametric tests depending on the distribution of the variables. For the independent samples, the Student's t-test and Mann-Whitney U test were used for comparing mean and median values, respectively. The correlations between variables were performed by Spearman's correlation test. Differences were considered significant at a probability level of $p<0.05$.

RESULTS

This study was performed on 36 HT patients and 36 controls. Demographic and clinical characteristics of the HT subjects and controls are shown in Table 1. PNX-14 and PNX-20 values were very significantly lower in HT patients compared with the control group ($p<0.001$). Spearman's rho correlation analysis was performed (Table 2). In HT group, the levels of PNX-14 were negatively correlated with weight ($r=-0.376$; $p<0.005$), BMI ($r=-0.407$; $p<0.001$), systolic BP ($r=-0.586$; $p<0.001$), and diastolic BP ($r=-0.319$; $p<0.01$). There was a negative correlation between serum PNX-20 and weight ($r=-0.378$; $p<0.005$), BMI ($r=-0.383$; $p<0.005$), systolic BP ($r=-0.551$; $p<0.001$), and diastolic BP ($r=-0.306$; $p<0.01$) in HT group.

We used receiver operating characteristic curve (ROC) analyses to compare the diagnosis value of PNX-14 and PNX-20 levels in HT patients (Figure 1). We, therefore, tested whether the predictive value of PNX-14 and PNX-20 were equal or superior by using ROC. We found that PNX-14 value is an area under the curve (AUC) of 0.87 (cutoff value 404.7 ng/L, sensitivity 92%, specificity 72%) and PNX-20 value is an AUC of 0.83 (cutoff value 209.9 ng/L, sensitivity 86%, specificity 75%). PNX-14 nearly showed equally compared to PNX-20 in predicting HT.

DISCUSSION

In this study, we show that in the HT group, serum PNX-14 and PNX-20 levels are significantly lower than that of healthy controls. In HT group, the levels of PNX-14 and PNX-20 were negatively correlated with weight, BMI, systolic BP, and diastolic BP. These findings suggest that serum PNX-14 and PNX-20 may be a potential biomarker in predicting the risk of HT.

Alteration in energy expenditure or metabolism plays important role in onset and course of HT. Central control of feeding behavior plays an essential role in metabolic homeostasis. PNX peptide was detected in the brain areas involved in controlling appetite[6]. Previous studies showed that IR may be involved in the pathogenesis of HT[14]. Pancreatic alpha and beta cells produce

and release glucagon and insulin, which differentially modulate the homeostasis of lipids and glucose. There is evidence that the biology of these cells may be modulated by PNX. The results showed that PNX stimulates insulin expression and secretion and promotes proliferation of INS-1E cells. PNX may contribute to the modulation of energy homeostasis and metabolism by controlling the neogenesis and secretion of insulin[7]. Several studies found that the blood PNX level depends on body mass[15]. In addition, PNX-14 increases the proliferation and differentiation of preadipocytes and decreases cell death, which indicates its possible role in the control of body mass regulation[10]. In this study, the levels of PNX-14 and PNX-20 were negatively correlated with weight, BMI, systolic BP, and diastolic BP. The PNX-14 and PNX-20 levels are significantly lower in HT patients than control groups and it could be a risk factor for obesity-associated HT. Furthermore, PNX-14 significantly ameliorated HFD-induced obesity and fatty liver[16]. Due to the limited numbers of studies concerning the effects of PNX on body mass, these results are difficult to interpret. Therefore, this association should be further investigated to also identify possible confounding factors.

Table 1. Clinical and demographic characteristics of hypertension and control subjects.

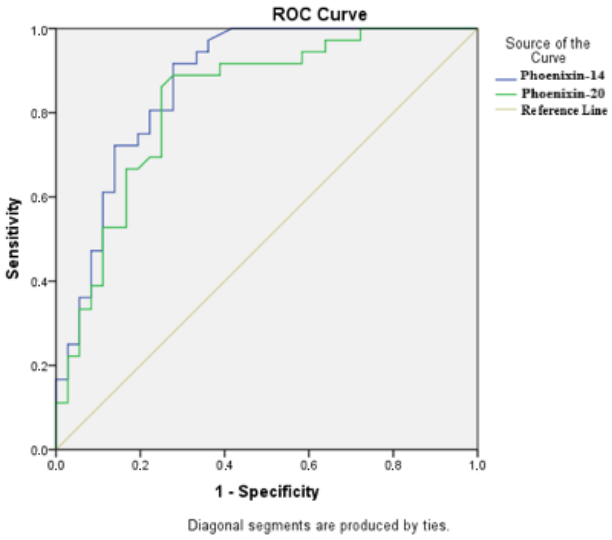
	Hypertension subjects n=36	Controls n=36	p
Age (years)	55±10.9	53±7.9	0.248
Female/male	14/22	18/18	0.285
Urea, mg/dL	29.0 (18–77)	26.5 (16–45)	0.073
Creatinine, mg/dL	0.76±0.16	0.82±0.17	0.133
AST, U/L	18.0 (12–56)	17.5 (9–27)	0.429
ALT, U/L	17.0 (8–154)	20.0 (3–41)	0.363
Fasting glucose, mg/dL	95 (78–152)	94 (71–107)	0.101
Triglycerides, mg/dL	162.4±82.9	148.7±61.1.9	0.425
HDL-C, mg/dL	51.9±12.0	53.1±10.5	0.662
LDL-C, mg/dL	136.8±40.5	128.8±26.2	0.327
Cholesterol, mg/dL	217.6±35.9	212.1±32.3	0.504
Potassium	4.5±0.4	4.4±0.2	0.94
Sodium	141 (137–145)	140 (136–145)	0.161
TSH, uIU/mL	1.4 (0.3–2.9)	1.7 (0.5–4.2)	0.157
Free $T_{4, ng/dL}$	1.3±0.2	1.3±0.1	0.393
Weight	85.5 (57–120)	66 (52–96)	<0.001
BMI, kg/m^2	31 (23–53)	24 (20–29)	<0.001
Systolic BP, mmHg	148 (120–200)	120 (110–160)	<0.001
Diastolic BP, mmHg	90 (70–130)	80 (70–90)	<0.001
Phoenixin-14, ng/L	335.4 (242.4–3196)	876.3 (356.4–3857.1)	<0.001
Phoenixin-20, ng/L	164.3 (110.4–2198.7)	285.2 (140.3–2476.8)	<0.001

AST: aspartate aminotransferase; ALT: alanine aminotransferase; BMI: body mass index; HDL-C: high density lipoprotein-cholesterol; LDL-C: low density lipoprotein-cholesterol; TSH: thyroid-stimulating hormone.

Table 2. Spearman's correlation analyses were performed to investigate the association of biomarkers levels in the hypertension subjects.

		Phoenixin-14	Phoenixin-20	Weight	BMI	Systolic BP	Diastolic BP
Phoenixin-14	r	1.0	0.905	-0.376	-0.407	-0.586	-0.319
	p	.	**<0.001**	**<0.005**	**<0.001**	**<0.001**	**<0.01**
Phoenixin-20	r	0.905	1.0	-0.378	-0.383	-0.551	-0.306
	p	**<0.001**	**.**	**<0.005**	**<0.005**	**<0.001**	**<0.01**

Bold value indicates statistically significant.



Area under the curve					
Test result variable(s)	Area	Std. error[a]	Asymptotic sig.[b]	Asymptotic 95% confidence interval	
				Lower bound	Upper bound
Phoenixin-14	0.873	0.042	0.000	0.790	0.955
Phoenixin-20	0.828	0.049	0.000	0.731	0.925

The test result variables Phoenixin-14 and Phoenixin-20 have at least one tie between the positive actual state group and the negative actual state group. Statistics may be biased. [a]Under the nonparametric assumption. [b]Null hypothesis: true area=0.5.

Figure 1. Phoenixin-14 and phoenixin-20 receiver operating characteristic curve.

HT is a multifactorial disorder associated with oxidative stress and inflammation[2,3]. The inhibitory effects of PNX on inflammation have recently been widely reported. Wang et al. reported that PNX-14 protected against lipopolysaccharide-induced inflammation in astrocytes[17]. Zeng et al. found that PNX-20 ameliorated lipopolysaccharide-induced inflammation in microglial cells[11]. The ability of PNX-14 to protect against oxygen-glucose deprivation/reoxygenation injury was also reported in human brain vascular endothelial cells[9]. Furthermore, since PNX-14 was detected at fairly high concentrations in the heart tissue, an involvement in cardiovascular functions has been hypothesized[4,8]. The role of PNX in ischemia/reperfusion (I/R) processes in the heart and in microglial cells of the brain was explored[8,18]. Rocca et al. showed that PNX administered at the reperfusion phase of I/R acted cardioprotectively[8]. PNX-14 inhibits I/R-induced cytotoxicity in microglia[18]. Another study showed that the myocardial injury and deteriorated cardiac function in diabetic mice induced by STZ were significantly ameliorated by PNX-14. In addition, the severe oxidative stress and inflammation in diabetic mice were dramatically mitigated by PNX-14[19]. The excessively released inflammatory factors and activated oxidative stress in gestational diabetes mellitus mouse model were alleviated by the administration of PNX-20[20]. These studies show that PNX exerts anti-inflammatory and cell protective effects. The development of HT is closely associated with inflammation and oxidative stress. According to these results, PNX exerts a possible beneficial effect against inflammation and oxidative stress in HT. In this study, we show that in the HT group, serum PNX-14 and PNX-20 levels are lower than that of healthy controls. Studies investigating the association of PNX with other inflammatory parameters in HT will provide further useful information to define its exact role in the pathogenesis of HT. Endothelium plays an important role in pathogenesis of many diseases and cardiovascular problems such as atherosclerosis and HT[21]. Wei et al. reported that GPR173 agonism by PNX-20 plays a protective role against ox-LDL-induced endothelial dysfunction[13]. VSMCs are major components of the vascular wall and serve to mediate hemodynamic vessel functions, playing a crucial role in regulating blood pressure in physiological conditions and HT. Under pathological conditions, VSMCs undergo differentiation, contractile, proliferative, and migratory alterations. These changes disrupt the function of vessels and can contribute to disease progression[22]. It was demonstrated that PNX-14 regulated proliferation and apoptosis of ox-LDL-treated VSMCs by modulation of the KCNQ1OT1/miR-183-3p/CTNNB1 axis[12]. These anti-inflammatory and antioxidative properties, such as regulatory effect in cell proliferation, apoptosis, and prominent role in controlling energy homeostasis and metabolism, may contribute to the prevention of HT.

These in vitro and in vivo results support to a potentially significant role for PNX in the control of BP. Our findings are consistent with previous studies, which implicated metabolic and inflammatory changes involved in the pathogenesis of HT.

Our study suggests PNX-14 and PNX-20 can be used as an indicative biomarker of HT.

Limitation of the present study was the small sample size. Further large-scale studies are needed to establish these associations and determine the role of PNX in the pathogenesis of HT. This is the first study presenting data on association between serum PNX-14 and PNX-20 levels and HT. Absence of comorbidities in our patient group increased the power of our study.

CONCLUSION

Phoenixin-14 and phoenixin-20 may be related to the pathogenesis of HT. The results of this study point out the possible role of PNX-14 and PNX-20 as potential novel biomarkers for the prediction the risk of HT. This result will be beneficial for further identification and development of potential drug targets for metabolic diseases.

INFORMED CONSENT

Informed consent was obtained from all individuals included in this study.

ETHICAL APPROVAL

The study was approved by the Local Ethics Committee Meram School of Medicine, Necmettin Erbakan University, Konya, Turkey (2021/3363).

AUTHORS' CONTRIBUTIONS

SA: Conceptualization, Data curation, Formal Analysis, Investigation, Writing – original draft, Writing – review & editing. **UC**: Conceptualization, Formal Analysis, Investigation, Writing – review & editing. **EP:** Conceptualization, Formal Analysis, Writing – review & editing.

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ORIGINAL ARTICLE

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The relation of dermcidin with insulin resistance and inflammation in women with polycystic ovary syndrome

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SUMMARY

OBJECTIVE: Polycystic ovary syndrome is the most common endocrinopathy among women of reproductive age. Polycystic ovary syndrome is a metabolic disorder associated with insulin resistance and subclinical inflammation. Dermcidin, an antimicrobial peptide, involves in insulin resistance and inflammatory processes. Dermcidin suppresses the secretion of insulin production from the liver/pancreas and also increases insulin resistance. We aimed to discover whether dermcidin levels were altered in polycystic ovary syndrome women compared to controls and determine the link of dermcidin with hormonal-metabolic parameters in polycystic ovary syndrome women.

METHODS: The current research was designed as a case-control study and Rotterdam 2003 criteria were used for diagnosing polycystic ovary syndrome. A total of 75 subjects with polycystic ovary syndrome and 75 age- and body mass index-matched subjects as controls were enrolled in the study. The insulin resistance state was determined using a homeostatic model assessment of insulin resistance and quantitative insulin sensitivity check index. High-sensitivity C-reactive protein levels were assessed to define inflammation.

RESULTS: Circulating dermcidin levels were measured by enzyme-linked immunosorbent assay. Dermcidin levels were significantly increased in polycystic ovary syndrome subjects compared to controls (172.53±42.41 ng/mL vs. 108.44±31.69 ng/mL, $p<0.001$). Homeostatic model assessment of insulin resistance and high-sensitivity C-reactive protein levels were markedly increased, whereas quantitative insulin sensitivity check index levels were notably decreased in women with polycystic ovary syndrome compared to controls. Linear regression analysis revealed that dermcidin exhibited an independent link with homeostatic model assessment of insulin resistance and high-sensitivity C-reactive protein, whereas dermcidin displayed an inversely independent link with quantitative insulin sensitivity check index.

CONCLUSION: Increased dermcidin levels were associated with insulin resistance and inflammation in polycystic ovary syndrome women, suggesting that dermcidin may play a role in the pathophysiology of polycystic ovary syndrome.

KEYWORDS: Polycystic ovary syndrome. Dermcidin. Insulin resistance. Inflammation.

INTRODUCTION

Polycystic ovary syndrome (PCOS) is a complex and heterogeneous disease, consists of both reproductive and metabolic issues, and affects 10–15% of women in their reproductive age. The disease is characterized by clinical and/or laboratory hyperandrogenism, menstrual dysfunctions, and polycystic ovaries. The pathophysiology of PCOS is not yet exactly clarified. A variety of mechanisms are considered to play a crucial role in the development of PCOS, including insulin resistance and inflammation. Insulin resistance and compensatory hyperinsulinemia affect up to 70% of women with PCOS. Besides insulin resistance, glucose and lipid metabolism disorders are common in women with PCOS, along with hypertension and obesity, and these metabolic and endocrinological disorders show variability with advancing age[1,2]. PCOS is associated with chronic low-grade inflammation. It has been reported that inflammatory markers such as C-reactive protein (CRP), pro-inflammatory cytokines, and chemokines are increased in women with PCOS[1-4]. Moreover, chronic low-grade inflammation has emerged as a key contributor to the metabolic and ovarian abnormalities, including androgen excess secretion in PCOS[1].

Dermcidin is an antimicrobial peptide that plays a crucial role in a variety of biological processes involving glucose metabolism and inflammation. Dermcidin induces insulin resistance. It was reported that dermcidin inhibited glucose uptake in the liver through the inhibition of glucose transporter type 4 (GLUT4) synthesis (Figure 1). GLUT4 is an insulin-regulated glucose transporter that mediates the uptake of glucose regulated by insulin[5]. In contrast, it was reported that dermcidin inhibited insulin secretion as well but the

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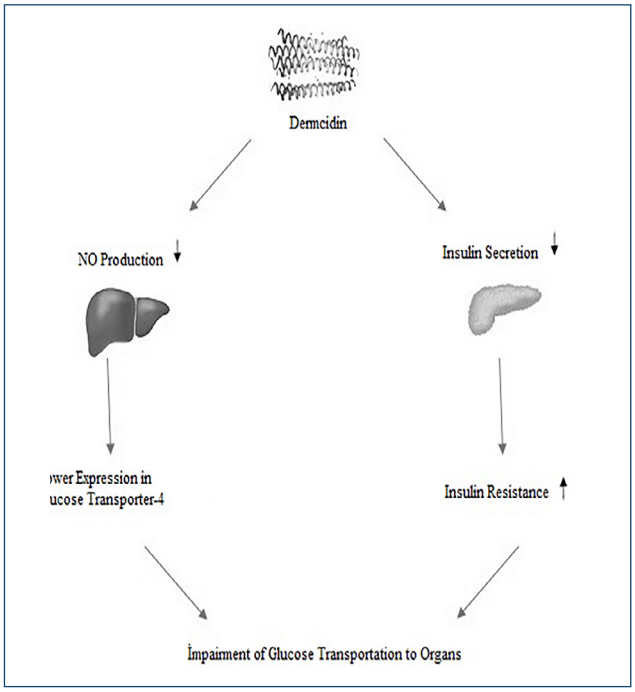


Figure 1. Effects of dermcidin on glucose metabolism.

mechanistic pathway has not yet been clarified. Additionally, it was demonstrated that dermcidin induces the synthesis of tumor necrosis factor-alpha (TNF-α)[6]. Clinical data are limited regarding dermcidin, and dermcidin levels were found to be elevated in subjects with hypertension, type 1 diabetes mellitus (T1DM), and gestational diabetes mellitus (GDM)[8-11]. Common treatment strategies in PCOS subjects are based on exercise, diet, nutrient supplementation, and treatment of insulin resistance[12]. Currently, insulin-sensitizing agents are also used to treat such patients based on their main pathophysiological substrate, hyperinsulinemia, but those treatment options are not sufficent. Therefore, we investigated dermcidin levels in women with PCOS[13].

We aimed to evaluate whether dermcidin levels were altered in women with PCOS compared to controls and evaluate whether there were any relationships between dermcidin and hormonal-metabolic parameters in women with PCOS.

METHODS

Ethics committee approval was obtained from Bozyaka Training and Research Hospital with decision number 29/05/2020-01. The written informed consent was taken from each recruited subject. The study was performed in accordance with the principles of the Declaration of Helsinki (revised in 2008).

A total of 75 PCOS subjects and 75 age- and body mass index (BMI)-matched subjects with normal menstrual cycle were recruited in this case-control study. The research was conducted between July 2019 and January 2020 in the Department of Internal Medicine, Bozyaka Training and Research Hospital.

Polycystic ovary syndrome group

Polycystic ovary syndrome subjects were selected using Rotterdam consensus criteria after excluding other causes of hyperandrogenism and ovulatory dysfunction. Although two out of three criteria are sufficient for choosing PCOS subjects, we had all the three following criteria in PCOS recognition for an appropriate homogeneity[10]: identification of oligo- and/or anovulation, identification of biochemical and/or clinical signs of hyperandrogenism, and use of the Ferriman-Gallwey [FG] for hirsutism determination[11] and occurrence of ≥12 follicles with the size of 2–9 mm in diameter or an ovarian volume of >10 mL (without a cyst or dominant follicle in either ovary) for determination of typical ultra-sonographic symptoms of polycystic ovaries as one ovary is sufficient for diagnosis. The subjects with FG score ≥8 were presented as hirsute. The biochemical hyperandrogenism was identified when testosterone (normal range: 0.52–2.42 nmol/l), and/or dehydroepiandrosterone sulfate (DHEA-S) (normal range: 10–248 µg/dl), and/or free androgen index (FAI) ≥5% of serum levels were more than the reference interval limitation[12].

Control group

The subjects were selected among women who visited gynecology or internal medical clinics for a routine checkup or who were volunteers to take part in the study by hospital employees. The subjects of the control group had normal menstrual cycles without concomitant health problems, acne, hyperandrogenism, or signs of hirsutism.

Exclusion criteria

Apart from PCOS, the subjects having the evidence of irregularity in menstrual cycles and/or excess androgen, i.e., hyperprolactinemia (<22 ng/mL), Cushing's syndrome (physical findings), non-classical congenital adrenal hyperplasia (17-hydroxy-progesterone <3 ng/mL)], thyroid disorders (0.41<TSH<4.5 µIU/mL), and galactorrhea, breastfeeding, pregnancy, decreased levels of glucose tolerance or having type 1/type 2 diabetes, familial hyperlipidemia, having a background of hypertension, suffering from liver/renal disorders or congestive heart failure, having a history of coronary artery disease, malignancy or acute infection (within 14 days), gestational diabetes mellitus, presence of any

chronic inflammatory or autoimmune disorders, undergoing treatment of hormonal contraception and/or anti-androgen (within the preceding 6 months), and not using medications for dyslipidemia, hypertension, hyperglycemia, insulin resistance, or obesity were excluded from the study.

Anthropometric evaluation

The weight (kg) and height (cmr) of subjects were measured. BMI was calculated using the following formula: BMI: weight (kg)/square meter of height (m^2). After a 15-min resting period, the blood pressure was measured while subjects were in a sitting position.

Biochemical evaluation

The venous blood samples of the subjects were obtained from the antecubital veins during the early follicular phase of menstrual bleeding (days 3–5), either spontaneous or progesterone-induced menses, in the morning following a 10-h fasting period. The blood samples were placed in room temperature for at least 30 min and allowed to clot. The clotted samples were centrifuged at 2000×g for 15 min. For analysis of neudesin, the separated serum samples were kept in aliquots at –80°C. Some dedicated kits (Abbott Diagnostics, Wiesbaden, Germany) from an auto-analyzer (Abbott Architect C 16000, IL, USA) were used to measure fasting plasma glucose (FPG), hs-CRP, total cholesterol, triglyceride, and high-density lipoprotein cholesterol (HDL-C). The low-density lipoprotein cholesterol (LDL-C) was calculated using the Friedewald equation[13]. Chemiluminescent microparticle immunoassay (CMIA) with its dedicated kits (Abbott Diagnostics, Wiesbaden, Germany) and auto-analyzer (Abbott Architect I2000, IL, USA) were used to measure serum fasting plasma insulin (FPI) levels. Luteinizing hormone (LH), follicle-stimulating hormone (FSH), estradiol (E_2), progesterone, DHEA-S, and total testosterone levels were also measured via CMIA (UniCel DXI 800, Beckman Coulter Inc., Brea, CA, USA). Additionally, the sex hormone-binding globulin (SHBG) level was measured via the chemiluminescence immunoassay technique (Immulite 2000 XPi, Simens Healthcare Diagnostics, Eschborn, Germany). The formula FAI: (total testosterone/SHBG)×100 was used to calculate FAI. Insulin resistance was measured via homeostasis model assessment of insulin resistance (HOMA-IR)[14] and quantitative insulin sensitivity check index (QUICKI)[15].

Measurement of circulating dermcidin by ELISA

Human enzyme-linked immunosorbent assay (ELISA) kits (Catalog number: 201-12-460, Sunred Bioscience, Shanghai, China) were used to measure dermcidin levels (in duplicate), following the instructions of manufacturer. Intra-assay coefficient of variability (CV) was <6% and the inter-assay CV was <8%. The level of circulation dermcidin range is between 1 and 300 ng/mL.

Statistical analysis

A power analysis using G Power 3.0.10 for Windows (Heinrich-Heine-Universität Düsseldorf, Düsseldorf, Germany) was considered to obtain the scale of population in the current study. The number of subjects involved in each group was calculated using this program. Regarding the abovementioned program, a group of 70 subjects was selected for each group as α and study power values were 0.05 and 0.90, respectively.

A Statistical Package for the Social Sciences software version 18.0 (SPSS Inc., Chicago, IL, USA) was used for statistical analyses. The distribution of variables was checked using Kolmogorov-Smirnov in each group. Normally distributed data were displayed as mean with standard deviation (SD). Student's t-test was used for the comparison of variables. Pearson's correlation coefficients were used to reveal the correlation of neudesin with metabolic and hormonal parameters. A multiple regression analysis was used to show an independent link between neudesin and correlated parameters. The level of 95% for the confidence interval was used. A two-sided $p<0.05$ was considered statistically significant.

RESULTS

Clinical and laboratory characteristics of the study population

The demographic and laboratory results are given in Table 1. Circulating dermcidin levels were significantly increased in PCOS subjects compared to controls (172.53±42.41 vs. 108.44±31.69 ng/mL, $p<0.001$). HOMA-IR, insulin, and hs-CRP levels were significantly higher in women with PCOS, whereas QICKI levels were significantly lower in women with PCOS than controls. Blood pressures did not differ between groups. Triglycerides were notably elevated, whereas HDL-cholesterol levels were remarkably lower in PCOS subjects compared to control. Total cholesterol and LDL-cholesterol levels did not show significant differences.

Correlation and multivariate regression analysis

Dermcidin levels showed a positive correlation with BMI, hs-CRP, HOMA-IR, and BMI, whereas it displayed a negative

Table 1. Comparison of the demographic and laboratory characteristics of the subjects.

Variables	PCOS (n=75)	Controls (n=75)	p[a]
Age, years	30.46±6.85	29.89±6.73	0.608
BMI, kg/m^2	26.60±4.48	26.87±4.51	0.709
SBP, mmHg	108.97±12.80	107.46±11.77	0.451
DBP, mmHg	74.28±6.60	73.61±5.73	0.506
Ferriman-Gallwey score	14.60±2.86	4.25±1.19	<0.001*
FBG, mg/dl	83.88±8.18	81.67±5.86	0.060
Insulin, µIU/ml	17.27±6.20	10.83±4.44	<0.001*
HOMA-IR	3.60±1.41	2.18± 0.89	<0.001*
QUICKI	0.32±0.01	0.34±0.02	<0.001*
Total cholesterol, mg/dl	208.08±33.64	201.57±43.72	0.309
LDL-C, mg/dl	137.97±28.75	130.94±27.58	0.129
HDL-C, mg/dl	41.52±9.77	48.79±10.89	<0.001*
Triglycerides, mg/dl	142.95±33.07	109.18±29.72	<0.001*
hs-CRP, mg/l	1.22±0.54	0.67± 0.21	<0.001*
FSH, mIU/ml	6.86±1.82	7.25±1.89	0.202
LH, mIU/ml	14.07±4.20	8.56±3.01	<0.001*
Progesterone, ng/ml	1.10±0.23	1.16±0.25	0.149
Estradiol, pg/ml	50.67±12.05	49.06±8.11	0.339
Total testosterone, nmol/l	2.90±0.41	1.69±0.35	<0.001*
SHBG, nmol/l	37.56±11.66	68.38±14.95	<0.001*
FAI, %	8.19±1.73	2.48±0.12	<0.001*
DHEA-SO$_4$, µg/dl	184.77±72.53	152.50±39.34	0.001*
Dermcidin, ng/ml	172.53±42.41	108.44±31.69	<0.001*

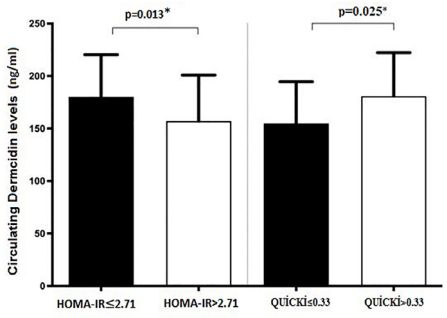
Results are given in mean±SD. [a]Independent samples Student's t-test was used. *A p<0.05 was considered significant. BMI: body mass index; DHEA-S: dehydroepiandrosterone sulfate; DBP: diastolic blood pressure; FAI: free androgen index; FBG: fasting blood glucose; FSH: follicle-stimulating hormone; HDL-C: high-density lipoprotein cholesterol; HOMA-IR: homeostasis model assessment of insulin resistance; hs-CRP: high-sensitivity C-reactive protein; LDL-C: low-density lipoprotein cholesterol; LH: luteinizing hormone; PCOS: polycystic ovary syndrome; QUICKI: quantitative insulin sensitivity check index; SBP: systolic blood pressure; SHBG: sex hormone-binding globulin.

correlation with QUICKI. Dermcidin levels exhibited a positive correlation with FBG, whereas dermcidin levels did not show a correlation with insulin levels. Moreover, dermcidin did not display any correlation with FSH, LH, estrogen, progesterone, and androgen as well as lipid profiles (Table 2). Dermcidin also showed an independent link with HOMA-IR and hs-CRP as well as an inversely independent link with QUICKI. Additionally, a positive correlation between dermcidin and BMI vanished in regression analysis (Table 2).

Comparing dermcidin levels in polycystic ovary syndrome subjects with and without insulin resistance using surrogate markers (quantitative insulin sensitivity check index and homeostasis model assessment of insulin resistance)

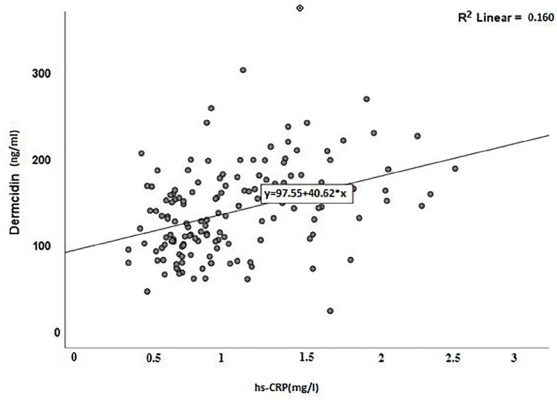
Polycystic ovary syndrome group was categorized into two different subdivisions according to the comprising insulin resistance (HOMA-IR>2.71 and HOMA-IR≤2.71 and QUICKI≤0.33 and QUICKI>0.33)[16,17]. Circulating dermcidin levels showed significant elevation in PCOS subjects having insulin resistance compared to those PCOS subjects not having insulin (Figure 2).

Since a statistically significant positive correlation was found between the dermcidin molecule and hs-CRP, mathematical modeling was performed to make an estimation according to the hs-CRP parameter by performing regression modeling, and the related equation is shown in Figure 3.



*Significance is defined as p<0.005.

Figure 2. Comparing dermcidin levels in PCOS.



*Significance is defined as p<0.005.

Figure 3. Predicting dermcidin with regression modeling according to hs-CRP.

Table 2. Pearson's correlation analysis and multiple regression analysis.

Variables	Correlation analysis		Multiple regression analysis			
	r	p	β	95%CI		p
				Lower	Upper	
Age	0.097	0.231	–	–	–	–
BMI	0.131	0.044*	0.053	-0.102	0.208	0.078
Insulin	0.098	0.064	–	–	–	–
FBG	0.287	0.012*	–	–	–	–
HOMA-IR	0.215	0.029*	0.214	0.091	0.337	0.019*
QUICKI	-0.197	0.034*	-0.159	-0.244	-0.074	0.038*
hs-CRP	0.162	0.041*	0.103	0.051	0.155	0.045*
FSH	0.045	0.267	–	–	–	–
LH	0.104	0.301	–	–	–	–
Estradiol	0.056	0.221	–	–	–	–
Progesterone	0.106	0.189	–	–	–	–
FAI	0.076	0.195	–	–	–	–
DHEA-S	0.085	0.206	–	–	–	–
Total cholesterol	0.112	0.087	–	–	–	–
LDL-C	0.058	0.228	–	–	–	–
HDL-C	-0.101	0.072	–	–	–	–
Triglycerides	0.109	0.065	–	–	–	–

Pearson's correlation analysis was used. *A $p<0.05$ was considered significant. Multiple linear regression analysis was used. β: unstandardized regression coefficient; CI: confidence interval; r: Pearson's correlation coefficient.

DISCUSSION

In the current study, we evaluated dermcidin levels in women with PCOS for the first time. We found that circulating dermcidin levels were significantly increased in PCOS subjects compared to controls. PCOS subjects with insulin resistance had higher levels of dermcidin than those PCOS subjects without insulin resistance. Dermcidin levels showed an independent link to HOMA-IR and hs-CRP, whereas dermcidin levels exhibited an inversely independent link to QUICKI.

Insulin resistance plays a key role in the pathophysiology of PCOS albeit the underlying cellular mechanisms are remaining unclear. In normal physiological conditions, insulin-stimulated glucose uptake mainly occurs through GLUT4 in muscle and fat tissues. It has been reported that GLUT4 protein expression is decreased in adipocytes of patients with PCOS[18]. Thus, the loss of GLUT4 in the adipocytes may be a significant contributor to the IR in patients with PCOS. Insulin receptor-mediated signal transduction is defective in women with PCOS as well[19]. Pre-clinical data suggest that dermcidin induces insulin resistance via inhibiting GLUT4 gene expression and subsequently dermcidin impairs glucose uptake in cells[6]. In the current study, we found that dermcidin levels were notably elevated in PCOS subjects. Consistently, we found that insulin resistance marker-HOMA-IR levels were increased, whereas insulin sensitivity marker-QUICKI levels were reduced in PCOS women. Moreover, we determined that dermcidin levels showed exhibited an independent link with HOMA-IR, whereas dermcidin displayed an inversely independent link with QUICKI. Additionally, PCOS subjects with insulin resistance had higher levels of dermcidin than those PCOS subjects without insulin resistance. In light of these data, dermcidin may play a role in the development of insulin resistance in PCOS subjects. Pre-clinical data suggest that dermcidin inhibits insulin secretion albeit we could not find any relation between dermcidin and insulin levels.

Polycystic ovary syndrome (PCOS) is associated with chronic low-grade inflammation. It has been reported that a variety of inflammatory markers in women with PCOS including CRP, tumor necrosis factor-α, and interleukin 6 are increased[1,2,20]. The etiology of systemic inflammation in PCOS remains unclear. Dermcidin plays a crucial role in

host immune defense. It has been reported that dermcidin induced TNF-α synthesis in liver cells[6]. In the current study, we found that circulating hs-CRP levels were higher in subjects with PCOS compared to controls. Moreover, we determined an independent link between dermcidin and hs-CRP. These data suggest that increased dermcidin may play a role in the development of inflammation in PCOS subjects.

Few clinical studies are currently involved in investigating the dermcidin levels; however, no data have yet been obtained regarding the relationship between dermcidin and metabolic parameters. A study showed that dermcidin plays a crucial role in the pathogenesis of T1DM as well as in the severity of the disease[6]. It was reported that dermcidin induced insulin resistance via inhibiting glucose uptake in the liver by decreasing GLUT4 synthesis. In addition, dermcidin induced inflammation[7,8]. In another study, circulating dermcidin levels in milk were notably elevated in subjects with gestational diabetes compared to controls[9].

We had some limitations in our study. Insulin resistance was evaluated using formulations instead of the insulin clamp technique, a gold standard but invasive method. Although this cross-sectional designed study does not provide causality, it allows discovering the link between molecules and disorders.

In conclusion, increased dermcidin levels were independently related to the degree of insulin resistance and inflammation in women with PCOS. Dermcidin may play a role in developing insulin resistance and inflammation in PCOS, which needs further research.

COMPLIANCE WITH ETHICAL STANDARDS

The subjects gave their oral and written informed consent before their inclusion in the study. The study adhered strictly to the principles of the Declaration of Helsinki as revised in 2008.

AUTHORS' CONTRIBUTIONS

OYA: Conceptualization, Data curation, Formal Analysis, Investigation, Methodology, Project administration, Resources, Software. **OB:** Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing.

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ORIGINAL ARTICLE

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COVID-19: the unmet need for family planning and its effects on sexuality: a cross-sectional study

Aysu Yıldız Karaahmet[1], Fatma Şule Bilgiç[1*]

SUMMARY

OBJECTIVE: This study was conducted to examine the effect of women's unmet family planning needs on their sexual functions during the COVID-19 pandemic period.

METHODS: A cross-sectional study was conducted with 319 women of childbearing age across Turkey between April and May 2021. Data were obtained through online questionnaires using the "Survey Form" and the Female Sexual Function Index.

RESULTS: It was observed that 46.77% of the participants had difficulty in accessing the family planning method, the most used family planning method during the pandemic period was the withdrawal method with 52.35%, and there was a significant difference between them and the pre-pandemic method ($p<0.05$). In the regression analysis, it was shown that a one-unit increase in the difficulty of accessing the family planning method and the place reached parameter would lead to an increase of 0.33 points in the sexual function probability of women.

CONCLUSIONS: It was observed that women of childbearing age living in Turkey had limited access to family planning services during the pandemic, those who used modern methods before the pandemic had to prefer the traditional method, and the sexual functions of women who had fear of becoming pregnant were adversely affected.

KEYWORDS: COVID-19. Unmet family planning. Sexual function. Woman health.

INTRODUCTION

The measures taken to stop and treat the COVID-19 epidemic and the increase in the burden on health systems caused some units to be closed or limited services to be provided. The pandemic process also affects sexual and reproductive health services and poses serious potential risks all over the world[1,2]. It has caused long-term disruptions in the provision of contraceptive products and services, especially in developing countries. Restriction of sexual and reproductive health clinics due to various reasons, restrictions on women's access to contraception and abortion, prevention of women's right to make autonomous decisions about their bodies and their lives, and, as a result, termination of unwanted pregnancies in unsafe environments are other possible problems[3,4].

Reproductive rights are a "human right" subject according to international and national definitions, and although individuals/families have the right to use them or not, some countries unfortunately consider birth control and induced abortion services within the group of deferrable services and especially among these services. Abortion is classified as "non-mandatory" and "non-medically urgent" care, and these countries are also implementing policies to effectively ban abortion within the scope of the COVID-19 outbreak[5,6]. These delays in reproductive health services have the potential to negatively affect the health of individuals and society in the longer term than the epidemic[3].

During the epidemic, people are expected to maintain social distance from people to reduce the risk of transmission. However, one of the behaviors that can be affected by social distance is sexual activity. In this period, when people distance themselves from each other due to social distance, there is a decrease in the frequency of sexual activity along with sexual reluctance[1,7,8]. During the COVID-19 pandemic, which threatens the whole world, some changes have been made in the access of reproductive health services. While the still unclear face of the epidemic and the anxiety caused by the prolongation of the process continue, the need for descriptive research on access to family planning and sexual health, which is the most important part of preventive health services, is increasing. This study was conducted to examine the effect of women's unmet family planning needs on their sexual functions during the COVID-19 pandemic period.

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METHODS

The STROBE Statement was used in the design, planning, implementation, and reporting of the cross-sectional, descriptive study. The study was carried out across Turkey between April and May 2021. The sample size was calculated as 344 people with the sample formula of unknown universe. The study was completed with 319 women at the scheduled time for data collection. For the research, post-hoc power analysis was performed using the G-Power version 3.1 statistical program. It was determined that for the chi-square test in a single group, a medium effect size of 0.30 and a significance level of 0.05, -0.996% power was reached.

Inclusion criteria

- Women aged between 18 and 49 years who were of childbearing age and accepted the study.
- Were literate and had internet access.
- Had an active sexual life.
- Volunteered to participate in the study.

Exclusion criteria

- Diagnosed sexual dysfunction.
- Diagnosed psychiatric disease.
- Antidepressant use.
- Experienced trauma in the last year.

Data were obtained through online surveys with women who met the sample selection criteria across Turkey.

Data collection tools

Data were obtained using the "Survey Form"[9-11] consisting of multidimensional questions and the "Female Sexual Functions Index" (FSFI).

Survey Form was created with a total of 21 questions, 4 of which were about the sociodemographic characteristics of women, 3 about their obstetric history, 4 about their sexual behavior in the COVID-19 period, and 10 questions about the family planning characteristics in the COVID-19 period.

FSFI is a 19-item Likert-type scale that evaluates the sexual dysfunction of women in the last 4 weeks. Its validity and reliability were determined by Rosen et al. (2000). The Turkish validity and reliability analysis of the scale was performed by Aygin and Aslan in 2005[12]. The Cronbach's alpha coefficient of the scale, which was adapted into Turkish, was found to be 0.95, and the test-retest reliability was between 0.75 and 0.95. This scale has six sub-dimensions: desire, arousal, lubrication, orgasm, sexual satisfaction, and pain, and a higher score from the scale means better sexual function. In this study, the internal consistency of the question items and a Cronbach's alpha coefficient for each item were calculated. Cronbach's alpha value is 0.83. Cronbach's alpha values for FSFI sub-dimensions were as follows: alpha desire: 0.723, arousal: 0.911, lubrication: 0.818, orgasm: 0.922, satisfaction: 0.787, and pain: 0.952. As a result of the factor analysis on the items, the adequacy of the sample and the sphericity of the data were found to be significant (KMO: 0.829, $\chi^2(78)=1360.94$; Bartlett test of sphericity (p)=0.000). The fact that the results are similar shows that the data were collected online and that the women reflected the situation while giving the answers.

Evaluation

The data were evaluated with the statistical package program (Statistical Package for the Social Sciences for Windows SPSS version 21.0) in computer environment. Results from descriptive statistics were presented as mean, number, and percentage. In the comparative analyses, independent t-test was used for normal distributions, and Mann-Whitney U, two-way analysis of variance, and multiple regression analysis were performed for non-normal distributions. Statistical significance level was accepted as $p<0.05$.

Ethical aspect of research

Ethics committee approval was obtained for the study from the non-interventional clinical research Ethics Committee (dated March 14, 2021, and numbered E.4646). The principles of the Declaration of Helsinki were complied with in the study. An informed consent form was added to the first page of the online questionnaires and the women were informed about the purpose and confidentiality of the research. Women who signed the "I agree" option at the end of the first page accepted that their consent was obtained and could proceed to the next page, while those who chose the "I do not agree" option were not allowed to answer the survey.

RESULTS

A total of 319 women with mean age 31.12±8.05 years, number of pregnancies 1.25±1.818, time of marriage years 3.72±1.20, and number of births 1.14±1.2 were included in the study. In addition, 49.21% of the participants had a university or higher education level, 81.8% did not work in any job, 58.0% had medium socioeconomic status, 89.0% lived in a nuclear family, and 38.9% never gave birth (Table 1).

Table 1 presents a comparison of information on family planning and sexual intercourse status of women before and after COVID-19. When we look at the frequency of sexual intercourse after COVID-19, it is seen that there is a decrease in

the frequency of sexual intercourse (p=0.0034) (Table 1). Table 2 shows the analysis of the relationship between the continuous variables of women's sexual function status. According to the analysis, there is a weak and negative relationship between total FSFI score and age, number of pregnancies, and years of marriage (r: -0.119, -0.043, -0.074) (p≤0.01) (Table 2).

Table 1. Comparison of participant's responses about family planning and sexual relationship status before and after the COVID-19 pandemic.

	Before COVID-19 (%)	After COVID-19 (%)	p-value
FP method usage			
Yes	160 (50.2)	117 (36.67)	**0.002**
No	159 (49.9)	202 (63.32)	
Difficulty with FP access			
Yes	45 (14.11)	149 (46.77)	**0.004**
No	274 (85.89)	173 (54.23)	
Where FP is procured			
FHC	201 (63.0)	100 (31.34)	0.078
Hospital	36 (11.2)	29 (9.09)	
Private polyclinic	46 (14.4)	48 (15.04)	
Market/pharmacy	36 (11.3)	106 (33.22)	
Change in FP requirement			
Yes	10 (3.14)	114 (35.7)	**0.0032**
No	309 (96.86)	205 (64.3)	
Change in FP method use			
Yes	17 (5.3)	189 (59.24)	**0.0017**
No	302 (94.7)	130 (40.75)	
The method he uses as an FP			
IUD	10 (3.13)	9 (2.82)	**0.022**
Condom	75 (23.51)	67 (21.00)	
OCs	47 (14.73)	17 (5.32)	
Calendar	55 (17.24)	65 (20.37)	
Retraction	132 (41.37)	167 (52.35)	
Frequency of sexual intercourse			
None	0 (0.0)	13(4.07)	**0.034**
1 per week	78 (24.45)	110 (34.48)	
2 per week	157 (49.21)	133 (41.69)	
3 or more per week	84 (26.33)	63 (19.74)	
Satisfaction with sexual intercourse			
Yes	207 (64.89)	187 (58.62)	0.082
No	112 (35.10)	132 (41.37)	

FP: family planning; FHC: family health center; IUD: intrauterine device; OC: oral contraceptive. Bold indicates significant p-value.

In Table 3, multiple regression analysis was performed to determine some variables that may affect the FSFI, namely, sexual function, of women with unmet family planning during the COVID-19 period. In the regression analysis, FSFI was used as the dependent variable, and the family planning method used during the pandemic period, the place where the method was accessed, the difficulty in accessing, and the method change were included in the model. According to the results of the regression analysis, when the significance level corresponding to the F-value is considered, it is seen that the established model is statistically significant. Four independent variables explain 3.3% of the variance in the dependent variable and the regression model is statistically significant (p<0.005). FP usage change in the COVID-19 period and required FP method status parameters had no significant effect on the model (p>0.05). In contrast, the difficulty in accessing FP methods and the parameters of the place available had a significant effect (p<0.005) in the COVID-19 period, which means that the difficulty in accessing the FP method and a one-unit increase in the reach parameter of the women's sexual orientation. It shows that it will lead to a 0.33 point increase in the probability of the function (Table 3).

DISCUSSION

The isolation process brought by COVID-19 may disrupt daily routines such as commuting to work, which takes half or sometimes the whole of the day, but it seems that this isolation process may allow people to spend more time with their partner and thus experience sexual activity more regularly[13-15]. But the quarantine measures were taken during the epidemic. It is possible to say that it negatively affects sexual relations. In general, it is stated in the studies that both sexual activities and sexual satisfaction of men and women decrease during the COVID-19 epidemic[16,17].

During the pandemic period, when people distanced themselves from each other due to social distance, there is a decrease in the frequency of sexual activity along with sexual reluctance. In a study conducted with 868 people in England, it was reported that 60.1% of the participants were not sexually active during self-isolation/social distancing. It was stated that the number of sexual activity was significantly higher in men than in women (p=0.002). Variables that are significantly associated with sexual activity are being male and young, being married or living with a family, consuming alcohol, and increasing days of isolation[7]. In a study conducted in China, it was stated that 25% of the participants experienced a decrease in sexual desire, 44% had a decrease in the number of sexual partners, and men had

Table 2. Relationship between women's continuous variables and female sexual functions index.

	Mean±SD	FSFI		Request		Arousal		Lubrication		Orgasm		Satisfaction		Pain	
		r	p	r	p	r	p	r	p	r	p	r	p	r	p
Age (years)	31.12±8.05	-0.175	**0.002**	-0.175	**0.002**	-0.098	**0.024**	-0.047	**0.041**	-0.018	0.075	-0.082	**0.014**	-0.146	**0.009**
Marriage time (year)	3.72±1.20	-0.074	**0.020**	-0.156	**0.005**	0.464	**0.014**	-0.052	**0.036**	-0.010	0.085	-0.042	**0.046**	-0.030	0.059
Number of pregnancy	1.25±1.81	-0.043	**0.045**	-0.072	**0.023**	-0.019	0.079	-0.052	**0.036**	0.026	0.065	-0.073	0.019	-0.011	**0.050**
Number of births	1.14±1.20	-0.007	0.090	-0.116	**0.039**	-0.019	0.073	0.080	**0.016**	0.074	**0.019**	-0.017	0.076	-0.033	0.055
Number of children	1.13±1.18	-0.011	0.084	-0.119	**0.034**	-0.020	0.072	0.082	**0.014**	0.066	**0.024**	-0.024	0.069	-0.030	0.059

FSFI: female sexual functions index; r: pearson. Bold indicates significant p-value.

Table 3. Multiple regression analysis of female sexual health status by characteristics related to family planning method use in the COVID-19 period.

Dependent variable	Independent variable	β	Standard error	β	t	p	VIF	F	Model (p)	R^2	Durbin Watson
FSFI	Constant	31.969	14.343	-	2.229	0.027*	-	3.541	**0.008***	0.033	1.989
	Change in FP method use in the COVID-19 period	1.366	4.882	.016	0.280	0.051*	1.062				
	Change in need for FP method use in the COVID-19 period	-4.034	2.224	-0.105	-1.814	0.071*	1.023				
	Difficulty in accessing the FP method in the COVID-19 period	14.150	6.131	0.136	2.308	0.022*	1.069				
	The place reaching the FP method in the COVID-19 period	1.965	0.824	0.135	2.348	0.020*	1.026				

FSFI: female sexual functions index; VIF: variance inflation factor. Bold indicates significant p-value.

a lower decrease in the number of sexual partners than women (53% vs. 30%). In addition, 32% of men and 39% of women stated that they also experienced a decrease in sexual satisfaction, and most of the participants with a history of risky sexual experiences also stated that risky sexual behavior acts decreased after the COVID-19 epidemic[18]. In this study, it was seen that there was a significant relationship between the fear of becoming pregnant during the COVID-19 period and the FSFI scores of the participants (p=0.005), and the FSFI score averages of the participants who were not afraid of becoming pregnant were higher. In a study examining the sexual functions of women during the pandemic, it was reported that while 32.7% of the participants wanted to become pregnant before the pandemic, this rate decreased by 5.1% during the pandemic (p=0.001). In the same study, it was determined that the use of contraception during the pandemic decreased significantly among the participants compared to the previous one[19]. Different findings on the subject have been found in the literature. This suggests that sexuality is multifactorial and affected by many factors.

In order not to increase the burden of unwanted pregnancies on the health system, it is necessary to continue the provision of family planning consultancy services as much as possible during the pandemic process[17,20]. The United Nations Population Fund (UNFPA) states that during the current pandemic period, more than 47 million women may not have access to modern family planning methods, and therefore, significant unwanted pregnancies may ocur[4]. In this study, it was seen that there was a significant relationship between having difficulty in reaching the family planning method in COVID-19 and FSFI scores, and the FSFI scores of the participants who had no difficulties in reaching it were higher. In the research, it was determined that the risk of infection transmission in health institutions decreased, the applications to family planning services decreased, there was a significant

decrease in the use of modern family planning during the pandemic process, and there was more disruption in family planning counseling in the public sector compared to the private sector[21-23]. The regression analysis performed in this study showed that difficulty in accessing the family planning method would lead to a 0.33 point increase in the probability of female sexual function. In a study conducted in India, there was a 36% decrease in the use of injectable birth control methods during the pandemic process, and a 21% decrease in the insertion of an intrauterine device (IUD). Similarly, combined oral contraceptive use decreased by 15% and condom use by 23%. These figures have been an indicator of family planning services that could not be met during the pandemic process in the country[11]. In cases where women apply to health institutions (e.g., pregnancy termination and delivery), effective family planning counseling should be given before discharge. Reproductive health services, which will affect the health of individuals and society in the long term, should be carried out in a controlled manner. In the pandemic process, practices such as providing individuals with access to family planning methods, continuing to provide services such as counseling and curettage in isolated centers, and providing consultancy with distance education are recommended to prevent the long-term public health problems[6,22,23]. With the results of the research, it was thought that access to family planning was reported as a problem, as in many countries in the world. Providing birth control methods and counseling to all men and women who request it is an important step in overcoming the pandemic process with the least damage.

CONCLUSIONS

The COVID-19 pandemic lockdown has disrupted access to contraceptives in Turkey, thus changing the dynamics of the unmet need for family planning. Individuals who initially used a modern method of contraceptive now lack access to their contraceptive method of choice for several reasons, including the fear of visiting health care facility, shutdown of drug/chemist stores, restriction of movements, and lack of access to health care providers. As a result, it was observed in the study that women of childbearing age living in Turkey restricted their access to family planning services during the pandemic and the sexual functions of women who were afraid of becoming pregnant were negatively affected.

AUTHORS' CONTRIBUTIONS

AYK: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Visualization, Writing – original draft. **FŞB:** Data curation, Investigation, Resources, Software, Validation, Visualization, Writing – review & editing.

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ORIGINAL ARTICLE

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Extended-spectrum beta-lactamases among *Klebsiella pneumoniae* from Iraqi patients with community-acquired pneumonia

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SUMMARY

OBJECTIVE: Beta-lactams resistance is a major clinical problem in treating pneumonia. This study aimed to detect the extended-spectrum beta-lactamases (ESBL) genes in *Klebsiella pneumoniae* among patients with community-acquired pneumonia (CAP) in Al-Najaf City, Iraq.

METHODS: A total of 511 sputum samples were obtained from all suspected patients with CAP in Al-Najaf City, Iraq, from March 2020 to September 2020. Sputum samples were subjected to microbiological tests. The disk diffusion method was used to test antibiotic sensitivity. Production of ESBLs was identified using phenotypic and genotypic methods.

RESULTS: The total prevalence of *K. pneumoniae* was 31.9% (163/511). Using CHROM agar, 41 (25.2%) isolates were ESBL producers. The imipenem 0.0% (n=0/41) and norfloxacin 0.0% (n=0/41) were the most effective antibiotics. The multiplex polymerase chain reaction showed that 46.3% (n=19/41) of isolates harbored ESBL genes. Out of 19 ESBL producers, 47.4% and 15.8% harbored bla_{CTX-M} and bla_{SHV}, respectively. While bla_{CTX-M} and bla_{SHV} genes were detected in 7 (36.8%) isolates, simultaneously.

CONCLUSIONS: The imipenem and norfloxacin can be used in empirical treatment of *K. pneumoniae* isolates in Iraq. The emergence of *K. pneumoniae* strains harboring ESBL resistance genes necessitates the development of a regular surveillance program to prevent the spreading of these isolates more in Iraqi health care systems.

KEYWORDS: bla_{CTX-M}. CAP. ESBL. Pneumonia. Klebsiella pneumoniae.

INTRODUCTION

According to the British Thoracic Society, community-acquired pneumonia (CAP) is an acute symptomatic infection of the lung parenchyma that occurs outside a hospital or nursing home[1]. CAP is caused by various microorganisms including *Klebsiella pneumoniae*[2]. No exact information about the incidence of the CAP in Iraq has been found so far. Clinical burden of CAP in older adults has only been assessed by a few large databases, with incidence rates ranging from 7.6 to 13.4 per 1,000 individuals[3]. A previous study from Iraq revealed *K. pneumoniae* as the leading cause of pneumonia[4].

Strains of *K. pneumoniae* that can produce extended-spectrum beta-lactamases (ESBLs) become seriously active against many types of beta-lactam antibiotics. In addition, these virulent strains are capable of becoming resistant to numerous classes of non-beta-lactams, making it difficult to treat infections, and are referred to as multidrug-resistant (MDR) strains[5].

Nearly 450 forms of ESBLs enzymes have been documented worldwide, and among these types, bla_{SHV}, bla_{TEM}, and bla_{CTX-M} were predominant. ESBLs are enzymes that contribute to resistance to a variety of beta-lactams[6]. ESBLs hydrolyze the beta-lactam ring of beta-lactam antibiotics, causing these antibiotics to lose their antimicrobial activity. These factors may contribute to the development of pneumonia complications[6,7].

To date, there are no studies on the prevalence of ESBL-producing *K. pneumoniae* in patients with CAP in Iraq. Therefore,

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the present research attempted to identify the ESBL-producing *K. pneumoniae* in Iraqi patients with CAP by phenotypic and molecular genotypic methods.

METHODS

Sample collection and bacterial isolation

The sputum samples of suspected patients suffering from CAP referred to Al-Sader Teaching Hospital in Al-Najaf City, Iraq, from March 2020 to September 2020 were collected in sterile containers. All patients were selected and diagnosed by a respiratory infectious disease specialist based on clinical examination and radiological and laboratory findings. Sputums were processed within 1 to 2 h of collection, using standard microbiological procedures. Sputums were initially cultured on blood agar and MacConkey agar (Merck, Germany) and incubated at 37°C for 2 days. The suspected *K. pneumoniae* colonies were further tested and identified using a panel of appropriate biochemical tests, including citrate utilization, urease, methyl red/Voges Proskauer, and triple sugar iron agar[8]. The confirmed *K. pneumoniae* isolates were stocked in tryptic soy broth containing 20% glycerol and placed at -80°C for long preservation.

Phenotypic detection of ESBL-producing *K. pneumoniae*

CHROM agar

All *K. pneumoniae* isolates were streaked on plates of CHROMagar™ ESBL agar (Pioneer, France). Chrome agar plates were aerobically incubated at 35°C overnight. Colonies of ESBL producers were appeared greenish blue.

Antimicrobial susceptibility testing

The ESBL-producing *K. pneumoniae* isolates were tested for antimicrobial susceptibility testing (AST) using disk diffusion technique according to the Clinical and Laboratory Standards Institute (CLSI) instructions[9]. Antimicrobials were classified as follows: aztreonam (ATM, 30 µg), gentamicin (CN, 10 µg), ciprofloxacin (CIP, 5 µg), ceftazidime (CAZ, 30 µg), levofloxacin (LEV, 5 µg), amoxicillin/clavulanate (AMC, 30 µg), trimethoprim (TMP, 5 µg), norfloxacin (NOR, 10 µg), cefotaxime (CTX, 30 µg), nitrofurantoin (F, 300 µg), imipenem (IPM, 10 µg), chloramphenicol (C, 30 µg), tetracycline (TE, 30 µg), and ceftriaxone (CRO, 30 µg) (Bioanalyse, Turkey). MDR isolates were determined according to the previous definition (resistance to at least one member of three antibiotics classes)[10]. *Escherichia coli* ATCC 25922 and *K. pneumoniae* ATCC 700603 were used as quality control strains.

Molecular detection of ESBL genes among *K. pneumoniae*

The presence of ESBLs encoding genes (bla_{SHV} and bla_{CTX-M}) were investigated by multiplex polymerase chain reaction (M-PCR) using previously described primer pairs (Bioneer, Koria)[11]. The DNA was extracted using genomic DNA extraction kit (FavorPrep, USA), according to the supplier instructions. All the components of M-PCR were mixed in final volume of 20 µl as follows: 12.5 µl of Master Mix (iNtRON, Koria), 5 µl of DNA template, 1.5 µl of DNA/RNA free water, and 0.5 µl of each reverse and forward primer. M-PCR mixture was put in a thermocycler (Biosystems, USA) instrument with following program: initial denaturation at 94°C for 5 min, 35 cycles of denaturation at 94°C for 50 s, annealing at 50°C for 40 s, elongation at 72°C for 60 s, and final extension at 72°C for 5 min. *E. coli* NCTC 13353 and *K. pneumoniae* ATCC 700603 were used as bla_{CTX-M}- and bla_{SHV}-positive controls, respectively.

Statistical analysis

The data for this research were analyzed statistically using the Statistical Package for Social Science (SPSS) version 20.0 (IBM Corp., Armonk, NY, USA).

RESULTS

Bacterial isolation

In total, 511 sputum samples were taken from 302 (59.1%) male and 209 (40.9%) female patients with CAP, from which 148 (29.0%) Gram-positive bacteria and 308 (60.3%) Gram-negative bacteria (GNB) were isolated. Also, 55 (10.7%) samples showed no bacterial growth. Out of 308 GNB, 163 (52.9%) isolates were identified as *K. pneumoniae* and were recorded as a major cause for pneumonia in this study. These isolates were obtained from 102 (62.6%) males and 61 (37.4%) females, respectively. The total prevalence of *K. pneumoniae* was 31.9% (163/511).

Phenotypic detection of ESBL producers

Using CHROM agar method, 41 (25.2%) and 122 (74.8%) *K. pneumoniae* isolates were found to be ESBL producers and non-ESBL producers, respectively.

Antibiotic susceptibility testing

This test was carried out on all ESBL-producing *K. pneumoniae* isolates against 14 antibiotics (Table 1). The ceftazidime

Table 1. An antimicrobial susceptibility testing of 41 extended-spectrum beta-lactamases producing isolates of *Klebsiella pneumoniae*.

Antibiotic agent	Number (%) of isolates		
	Resistance	Intermediate	Susceptible
Amoxicillin/clavulanate	18 (43.9)	0 (0.0)	23 (56.1)
Aztreonam	39 (95.1)	0 (0.0)	2 (4.9)
Cefotaxime	40 (97.6)	0 (0.0)	1 (2.4)
Ceftazidime	41 (100.0)	0 (0.0)	0 (0.0)
Ceftriaxone	38 (92.7)	0 (0.0)	3 (7.3)
Chloramphenicol	17 (41.5)	3 (7.3)	21 (51.2)
Ciprofloxacin	11 (26.8)	0 (0.0)	30 (73.2)
Gentamicin	19 (46.3)	3 (7.4)	19 (46.3)
Imipenem	0 (0.0)	0 (0.0)	41 (100.0)
Levofloxacin	9 (22.0)	2 (4.9)	30 (73.1)
Nitrofurantoin	9 (22.0)	0 (0.0)	32 (78.0)
Norfloxacin	0 (0.0)	0 (0.0)	41 (100.0)
Tetracycline	29 (70.7)	0 (0.0)	12 (29.3)
Trimethoprim	27 (65.9)	0 (0.0)	14 (34.1)

(n=41/41; 100%), cefotaxime (n=40/41; 97.6%), ceftriaxone (n=38/41; 92.7%), and aztreonam (n=39/41; 95.1%) were among the less effective antibiotics, while imipenem (n=0/41; 0.0%) and norfloxacin (n=0/41; 0.0%) were the most effective antimicrobials. In total, 27 (65.9%) ESBL-producing *K. pneumoniae* isolates were MDR due to the resistance to at least one member of three antibiotics classes.

Molecular detection of ESBLs encoding genes

The M-PCR showed that 46.3% (n=19/41) of isolates harbored ESBL genes, while 53.7% (n=22/41) of isolates were found to be negative for these genes. Out of total 19 ESBL-positive isolates, 47.4% (n=9) harbored bla_{CTX-M} and 15.8% (n=3) harbored bla_{SHV} genes. And, bla_{CTX-M} and bla_{SHV} genes were detected in 7 (36.8%) isolates simultaneously. The bla_{CTX-M} was the most dominant gene and present either alone or in combination with bla_{SHV} gene.

DISCUSSION

In this study, the bacterial isolates were obtained from 89.2% (n=456/511) of the CAP patients, which was significantly higher than those obtained in the studies by Kishimbo et al.[1] from Tanzania (20.4%) and Regassa[12] from South Ethiopia (42.9%). These discrepancies may be due to differences in the study population, sample size, and geographical variations. This study showed more prevalence of CAP in male patients than females, which was consistent with previous report from Australia[13].

In this study, the total prevalence of *K. pneumoniae* was 31.9% among patients with CAP. This finding was lower than the previous study (54.0%) by Jaaffar et al.[4] from Iraq and higher (18.0%) than the former studies by Temesgen et al.[14] from Ethiopia.

In this study, the ESBL-producing *K. pneumoniae* showed high resistance rates against ceftazidime (100.0%), cefotaxime (97.6%), aztreonam (95.1%), ceftriaxone (92.7%), tetracycline (70.7%), and trimethoprim (65.9%), whereas all isolates were susceptible to imipenem and norfloxacin. These results were closely similar to those observed by Fils et al.[15] from France and Liu et al.[16] from China. In this research, *K. pneumoniae* isolates showed good susceptibility to fluoroquinolones and aminoglycosides. In line with our results, Zhang et al.[17] from China reported the good efficacy of ciprofloxacin and levofloxacin against *K. pneumoniae* causing community-onset infections.

Another finding of this study was the high frequency of 65.9% for MDR phenotype among ESBL-producing *K. pneumoniae* isolates. This finding was in parallel with the previous reports from Brazil (84.0%)[10] and Portugal (100%)[18]. Teklu et al.[19] concluded that the main explanation for these high resistance rates may be due to the widespread, excessive, irregular, unnecessary, and uncontrolled use of antibiotics to treat various infections. Carbapenems are still used as the best option to treat various infections, including pneumonia caused by ESBL-producing GNB. The results of this study were in agreement to the findings of most international studies, according to which imipenem has high efficacy against ESBL-producing *K. pneumoniae*[20-22].

In this study, 25.2% (n=41/163) of *K. pneumoniae* isolates showed phenotypic positive result for ESBL production using CHROM agar. Ultimately, this research was unable to validate the existence of ESBL genes by M-PCR in all isolates that had phenotypic positive test. It was found that 19 of 41 ESBL producers harbored ESBL genes using M-PCR method. These findings may presumably be due to the involvement of additional resistance pathways such as Ambler class C beta-lactamases, the presence of other mechanisms of resistance to beta-lactams, and the presence of other ESBL genes such as bla_{TEM} and bla_{PER} leading to differences between the results of phenotypic and molecular methods[23,24]. According to the CLSI, the combination disk test (CDST) is recommended for confirmation of ESBL production in Enterobacteriaceae using CAZ (30 µg) and CTX (30 µg) alone and in combination with clavulanic acid[9].

The results of this study showed that bla_{CTX-M} was the most common ESBL gene among *K. pneumoniae* isolates.

The worldwide spread of bla_{CTX-M} producing *K. pneumoniae* is a major concern in most continents. In a meta-analysis by Eskandari-Nasab et al.[25], the prevalence of bla_{CTX-M} was documented in Bahrain, Turkey, Saudi Arabia, Iran, United Arab Emirates, Pakistan, and Kuwait as 10.0, 30.0, 35.3, 56.7, 64.4, 96.9, and 100.0%, respectively. While international studies recorded varying percentages for the presence of this gene among the isolates producing ESBLs, including North Africa, America, Russia, Latin America, Brazil, and European countries, the percentages were 7.4, 26.4, 34.9, 61.1, 62.1, and 84.5%, respectively[25]. Despite the fact that TEM and SHV variants are the most universal ESBLs, it seems that they have become less common over the past decade than CTX-M. The results of this study were consistent with previous studies that found the bla_{CTX-M} gene as the most widespread ESBL type in *K. pneumoniae* isolates[16,17,25]. However, Ferreira et al.[10] from Brazil and Carvalho et al.[18] from Portugal showed a higher prevalence of bla_{SHV} compared to bla_{CTX-M} in *K. pneumoniae,* which was in contrast to our finding. Many factors, including the sample origin, sample size, studied population, and detection methods, can contribute to these differences.

Finally, our results showed the co-existence of ESBL genes in 36.8% of *K. pneumoniae* isolates. Previous studies from Brazil[10], China[16,17], and Portugal[18] reported the co-existence of various ESBL genes among clinical isolates of *K. pneumoniae*. This study had several limitations: the lack of screening of other ESBL genes such as bla_{TEM} and bla_{PER}, the lack of clinical data of patients to investigate the ESBL-related risk factors, and lack of sequencing for detected ESBL genes.

CONCLUSIONS

The emergence of MDR *K. pneumoniae* strains harboring ESBL resistance genes necessitates the development of a regular surveillance program to monitor, control, and prevent the more spread of these isolates in Iraqi health care systems.

AUTHORS' CONTRIBUTIONS

FEAR: Conceptualization, Data curation, Formal analysis, Writing – original draft, Writing – review & editing. **EB:** Conceptualization, Data curation, Formal analysis, Writing – original draft, Writing – review & editing. **MKA:** Conceptualization, Data curation, Formal analysis, Writing – original draft, Writing – review & editing. **SA:** Conceptualization, Formal analysis, Writing – original draft, Writing – review & editing. **MS:** Formal analysis, Writing – original draft.

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ORIGINAL ARTICLE

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C-reactive protein to lymphocyte count ratio is a promising novel marker in hepatitis C infection: the clear hep-c study

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SUMMARY

OBJECTIVE: Chronic hepatitis C (CHC) is one of the most important health problems affecting the significant rate of world population and it may lead to cirrhosis and hepatocellular carcinoma. C-reactive protein to lymphocyte count ratio (CLR) is used in estimating inflammatory burden. Therefore, this study aimed to compare CLR values between CHC patients and healthy controls and between CHC patients with and without fibrosis.

METHODS: Patients with CHC infection who visited outpatient and inpatient internal medicine clinics of our institution between January 2021 and December 2021 were enrolled to this retrospective study. CLR of the patients with CHC and healthy controls were compared. We further compared CLR of CHC patients with and without fibrosis.

RESULTS: Median CLR of CHC and control subjects was 2.61 (5.13%) and 0.31 (0.37%), respectively. CLR of the CHC group was significantly increased compared to the CLR of the controls (p<0.001). There was a significant positive correlation between CLR and APRI score (r=0.15, p=0.04). The sensitivity and specificity of CLR in determining CHC above 0.58% level were 84% and 82%, respectively (AUC: 0.884, p<0.001, 95%CI 0.84–0.93). In subgroup analysis, CLR was 3.97 (6.6%) for CHC patients with fibrosis and 1.7 (4.4%) for CHC subjects without fibrosis (p=0.001).

CONCLUSIONS: Increased CLR in patients with CHC may be an alarming finding of liver fibrosis, as CLR is associated with both CHC and hepatic fibrosis.

KEYWORDS: Inflammation. Chronic hepatitis C. Fibrosis. C-reactive protein to lymphocyte count ratio.

INTRODUCTION

Chronic hepatitis C (CHC) is one of the most important health issues affecting about 2–3% of the general population in the world[1]. CHC may cause liver cirrhosis and hepatocellular carcinoma[2,3]. Treatment of CHC is based on the data about the degree of the hepatic fibrosis, which is estimated by noninvasive aspartate-to-platelet ratio index (APRI) and FIB4 scores and by histopathological evaluation of the liver biopsy specimen, which is an invasive procedure[4,5]. To estimate fibrosis in patients with CHC, novel, cost-effective, and noninvasive markers are needed.

Reports in literature introduced a novel biomarker, C-reactive protein to lymphocyte count ratio (CLR), to estimate inflammatory burden in certain conditions. Preliminary CLR studies were on cancer and suggested that CLR could be a reliable marker of prognosis in a variety of malignant conditions[6,7]. CHC produces significant amount of inflammation as those malignancies do. Therefore, we designed this study to investigate the hypothesis that CLR may have a role in CHC and predict the degree of fibrosis. CLR values of the CHC patients were compared to those of healthy controls and also CHC patients with fibrosis to those without fibrosis.

METHODS

Study population

After study protocol was approved by institutional ethics committee (approval no: 2021/291, approval date: December 7, 2021), patients with CHC infection who visited outpatient and inpatient internal medicine clinics of our institution between January 2021 and December 2021 were enrolled in this retrospective study. Control subjects were healthy volunteers who visited our clinics for a routine checkup within the same time period. Subjects with malignancy, acute infection, and active inflammatory diseases were excluded from the study.

Anthropometric and laboratory analyses

Age and sex of the participants were recorded. White blood cell (WBC); neutrophil (neu), lymphocyte (lym), and platelet (PLT) counts; hemoglobin (Hb); hematocrit (Hct); aspartate (AST) and alanine (ALT) transaminases; blood urea; creatinine; and serum albumin levels were obtained from institutional database. Hemogram parameters were measured using the Abbott Cell-Dyn 3700 complete blood count device

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(Abbott Laboratories, Abbott Park, IL, USA). Serum biochemistry parameters were measured using an Abbott Architect C8000 auto-analyzer (Abbott Laboratories). APRI and FIB4 scores were calculated with the following formulas: [(AST/upper limit of the normal AST range) × 100]/PLT and [(age × AST) / (PLT × √ALT)], respectively. A CLR was calculated by dividing CRP by lym. Data of the patients with CHC and controls were compared. We further grouped CHC patients as fibrosis group and no-fibrosis group according to the HAI score in histopathological examination of liver biopsy. CLR and other variables of the fibrosis group and no-fibrosis group were compared.

Statistical analyses

Statistical analyses were conducted by using statistics software (SPSS version 16.0 for Windows; IBM Co., Armonk, NY, USA). Kolmogorov-Smirnov test was used to identify whether variables are normally distributed. Variables with normal distribution were compared with t-test and expressed as mean±standard deviation (SD). Mann-Whitney U test was used to compare non-normally distributed variables and those variables were expressed as median (interquartile range [IQR]). Categorical variables were compared with chi-square test and expressed as percentages and numbers. Correlation between CLR and other study variables was analyzed with Pearson's correlation test. The sensitivity and specificity of CLR and other variables in predicting CHC were obtained by receiver operative characteristics (ROC) test. Binary logistic regression analysis adjusted to age, AST, ALT, APRI, and FIB4 scores was performed to evaluate whether CLR was an independent risk factor for hepatic fibrosis in CHC patients. Statistical significance was considered at $p<0.05$.

RESULTS

A total of 198 subjects were enrolled in the study, with 132 in CHC group and 66 in control group. Median age was 55 (23) years for CHC group and 52.5 (5) years for controls (p=0.067). In CHC group, 68 (51.5%) were women and 64 (48.5%) were men, while 25 (38%) were women and 41 (62%) were men in control group (p=0.07).

Table 1 shows characteristics and laboratory data of the CHC and control groups. Median CLR of CHC and control subjects was 2.61 (5.13%) and 0.31 (0.37%), respectively. Therefore, CLR of the CHC group was significantly higher than that of the controls (p<0.001).

There was a significant positive correlation between CLR and APRI score (r=0.15, p=0.04).

The sensitivity and specificity of CLR in determining CHC above 0.58% level were 84 and 82%, respectively (AUC: 0.884, p<0.001, 95%CI 0.84–0.93). APRI score >0.23 with 81% sensitivity and 82% specificity indicates CHC (AUC: 0.871, p<0.001, 95%CI 0.82–0.92), while FIB4 score >1 with 75% sensitivity and 73% specificity indicates CHC (AUC: 0.807, p<0.001, 95%CI 0.75–0.87). Figure 1 shows the ROC curves of CLR, APRI, and FIB4 scores in determining CHC subjects.

We further compared CHC patients with fibrosis to those without fibrosis in histopathological evaluation. Age (p=0.42), WBC (p=0.4), neu (p=0.75), lym (p=0.22), Hb (p=0.06), Hct (p=0.06), PLT (p=0.21), ALT (p=0.22), urea (p=0.57), and creatinine (p=0.08) levels were not statistically different between CHC subjects with and without fibrosis. CRP (p<0.001), APRI score (p=0.002), FIB4 score (p=0.001), AST (p=0.007), albumin (p=0.01), and HAI score (p<0.001) of the CHC patients with and without fibrosis were significantly different from each other. CLR of the CHC patients with fibrosis was 3.97 (6.6%) and for those without fibrosis was 1.7 (4.4%), with statistically significant difference (p=0.001).

Table 1. Summary of the data of study population.

Variable	CHC group	Control group	p
Sex			
Male (n, %)	64 (48,5)	41 (62)	0.07
Female (n, %)	68 (51,5)	25 (38)	
Mean±SD			
Lym (k/mm^3)	1.9 ± 0.86	2.1 ± 0.61	0.104
Hb (g/dL)	13.5 ±2.2	14.6 ± 1.5	**<0.001**
Hct (%)	40.4 ± 6.5	44 ± 4.5	**<0.001**
PLT (k/mm^3)	199 ± 77	240 ± 44	**<0.001**
Median (IQR)			
Age (years)	55 (23)	52.5 (5)	0.067
CLR (%)	2.61 (5.13)	0.31 (0.37)	**<0.001**
CRP (mg/L)	4.6 (9.2)	0.7 (0.8)	**<0.001**
WBC (k/mm^3)	6.5 (2.5)	7 (3)	0.06
Neu (k/mm^3)	3.25 (1.5)	3.9 (1.6)	0.071
APRI (%)	0.53 (0.68)	0.19 (0.07)	**<0.001**
FIB4 (%)	1.79 (2.43)	0.87 (0.37)	**<0.001**
AST (U/L)	39.5 (42.5)	17 (5.3)	**<0.001**
ALT (U/L)	42 (51)	18 (8.5)	**<0.001**
Blood urea (mg/dL)	30 (15)	24.5 (11)	**<0.001**
Creatinine (mg/dL)	0.883 (0.38)	0.75 (0.12)	**0.002**
Serum albumin (g/L)	41 (7)	45 (1)	**<0.001**

Bold indicates significant p-value.

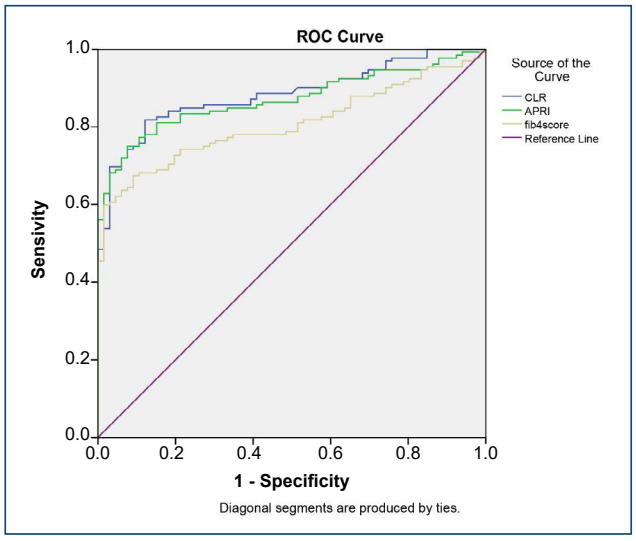

Figure 1. Receiver operating characteristic curves of C-reactive protein to lymphocyte count ratio, aspartate-to-platelet ratio index, and fibrosis-4 scores in determining chronic hepatitis C.

Binary logistic regression analysis adjusted to age, AST, ALT, APRI, and FIB4 scores showed that CLR was an independent risk factor for fibrosis in CHC patients (p=0.01, OR: 0.88, 95%CI 0.79–0.98).

DISCUSSION

Results of this study show that CLR could be associated with the presence of CHC and may be a marker of fibrosis in this population. In addition, CLR has significant correlation with APRI score, another predictor of fibrosis in chronic hepatitis subjects. Finally, we showed that CLR has better sensitivity and specificity than both APRI and FIB4 scores in determining subjects with CHC.

A study on CRP and lymphocyte count in heart failure patients found that CRP was increased and lymphocyte count was reduced (meaning elevated CLR) in heart failure compared to healthy individuals[8]. Moreover, a recent study in patients with incarcerated hernia reported increased CRP and decreased lymphocyte count were associated with intestinal ischemia in that population[9]. Thus, both intestinal ischemia and heart failure are associated with some degree of inflammatory burden. Similarly, CHC is associated with inflammation; thus, a similar elevation in CLR was found in CHC patients in this study.

Association between CLR and inflammatory conditions has been well established. In a study investigating the prognostic role of CLR in patients undergoing esophagogastric resection for esophageal cancer, it was found that CLR is a useful marker in the prediction of major morbidity after esophagogastric resection surgery[7]. A recent work that studied CLR in pancreatic cancer revealed that it was better than any other prognostic indicators in predicting survival of those patients[6]. Subsequently, their findings were supported in another study by Fan et al., which reported CLR as a useful prognostic marker[10]. Pancreatic cancer, like all malignancies, induces significant inflammation. In contrast, inflammation also plays an important role in surgical procedures. Since inflammation is a common pathway in those conditions and in CHC, this study also reported elevated CLR.

The prognostic role of CLR has been studied in other malignancies, too. According to a study which observed CLR in oral malignancy, elevated level of CLR was associated with better prognostic performance compared to other inflammatory markers in subjects with squamous cell carcinoma[11]. Similarly, Meng et al. investigated CLR in patients with colorectal cancer and reported that patients with high CLR had shorter overall survival than those with low CLR levels[12]. Subsequently, Mungan et al.[13] confirmed the results of Meng et al.[12]. In addition, prognostic value of elevated CLR has also been shown in patients with osteosarcoma[14], cholangiocarcinoma[15], and lung cancer[16]. It is a fact that malignant conditions are associated with increased inflammatory burden[17], as seen in patients with CHC. Therefore, increased CLR levels in CHC and further higher CLR in subjects with hepatic fibrosis, which reported in this study, are the findings consistent with literature knowledge.

Hepatic fibrosis in CHC is associated with increased inflammatory burden[18,19]. Hepatic stellate cells are responsible of accumulation of extracellular matrix proteins (i.e., collagen) in liver in patients with CHC. Inflammatory cytokines and chemokines which produced and released by hepatitis C-infected hepatocytes trigger the activation of hepatic stellate cells[20]. Indeed, we found higher CLR levels in CHC patients with fibrosis compared to the CHC subjects without liver fibrosis. Inexpensive, easy-to-assess, and noninvasive nature are advantages of CLR over other fibrosis markers in CHC.

There are three main limitations of this study: retrospective design, relatively small study cohort, and single-center nature of the conducted work. Due to single-center nature, the association between CHC and CLR may not be a global association. However, to the best of our knowledge, this is the first study in literature to report elevated CLR in CHC patients and even higher levels of CLR in those with liver fibrosis compared to the subjects without fibrosis.

CONCLUSIONS

Increased CLR in patients with CHC may be an alarming finding of liver fibrosis since CLR is associated with both CHC and hepatic

fibrosis. Therefore, inexpensive and easy-to-assess nature of CLR make it a useful marker in clinical follow-up of this population.

AUTHORS' CONTRIBUTIONS

MED: Conceptualization, Data curation, Writing – review & editing. **GA:** Conceptualization, Formal analysis, Supervision, Writing – original draft, Writing – review & editing. **SB:** Conceptualization, Methodology, Software. **GK:** Data curation, Writing – original draft, Writing – review & editing. **OK:** Data curation, Writing – original draft, Writing – review & editing. **BMA:** Data curation, Formal analysis, Writing – review & editing. **TTD:** Conceptualization, Data curation, Writing – review & editing.

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ORIGINAL ARTICLE

AOP

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Impact of coronavirus disease 2019 pandemic on breast cancer screening and detection of high-risk mammographic findings

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SUMMARY

OBJECTIVE: The coronavirus disease 2019 pandemic has disrupted cancer screening worldwide. This study aims to analyze the changes in the rates of screening mammograms and BIRADS 4 or 5 mammograms during the coronavirus disease 2019 pandemic in the opportunistic scenario.

METHODS: We integrated three different public databases from the state of São Paulo, Brazil, to obtain the rate of screening mammograms per 1,000, and the rate of BIRADS 4 or 5 mammograms per 100,000 women aged from 50 to 69 years in the years from January 2017 to December 2020.

RESULTS: The mean monthly screening mammograms decreased from 14.8/1,000 in 2019 to 9.25/1,000 in 2020, with the lowest rates being recorded in May 2020 (3.1/1,000). The mean monthly high-risk mammograms decreased from 12.8/100,000 in 2019 to 9.1/100,000 in 2020, with the lowest rates being recorded in April 2020 (4.3/100,000).

CONCLUSIONS: Coronavirus disease 2019 pandemic significantly decreased mammography screening in an opportunistic scenario, a warning sign for decreasing diagnosis of breast cancer in early stages, and increasing advanced stage diagnosis and mortality in the future.

KEYWORDS: Breast neoplasms. Early detection of cancer. COVID-19. Mammography.

INTRODUCTION

In December 2019, in Wuhan, China, an epidemy of a pneumonic viral disease named coronavirus disease 2019 (COVID-19) began. The novel coronavirus (SARS-CoV-2) presents rapid interpersonal spread and may cause severe acute lung injury[1,2]. In Brazil, the first COVID-19 case was reported in the city of São Paulo in March 2020[3]. Based on the international recommendations, the state government adopted social distancing measures and prioritized health resources to face the pandemic[4,5]. These policies and the general fear of the population in seeking healthcare have lowered breast cancer screening[6,7].

Interruption of mammographic screening, even for a short period, may result in lower diagnosis of early-stage cancers and a higher risk of mammographic findings in the future, leading to an overload on the healthcare system, with demands of diagnosis and treatment procedures[8,9]. The Brazilian Ministry of Health has an early detection breast cancer program for women aged from 50 to 69 years and recommends mammographic screening for every 2 years. The program is opportunistic; therefore, women are not invited to participate. They need to seek healthcare to have their examinations performed[10]. In this context, we proposed to evaluate the impact of the COVID-19 pandemic in the state of Sao Paulo, considering overall and high-risk finding (BI-RADS 4 and 5) rates.

METHODS

Study design

We performed a retrospective and descriptive study of public records, using data available in the following databases: Cancer Information System (SISCAN), The Brazilian Institute of Geography and Statistics (IBGE), and The Supplementary Health National Agency (ANS). We extracted data on the number of screening mammograms in the public health system (SUS) among women aged from 50 to 69 years, in each city of the state of São Paulo, from January 2017 to December 2020. In addition, we obtained the numbers of BIRADS 4 or 5 mammograms. We also obtained data of the female resident in each city and the female population with private health insurance. This manuscript follows the STROBE guideline.

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Ethics approval and consent to participate

Research that involves exclusively the public domain data and does not identify research participants does not need ethical approval. We followed the Declaration of Helsinki.

Data source and variables

Cancer Information System is a public database that contains information regarding all mammograms performed in the SUS. We extracted tables with the number of screening mammograms of women aged between 50 and 69 years, from January 2017 to December 2020, in all the cities of the state of São Paulo (http://tabnet.datasus.gov.br/).

The IBGE system contains public information about population census and expected population. We extracted tables with the female expected annual population, aged from 50 to 69 years, in all the cities of the state of São Paulo in the years from 2017 to 2020 (https://www.ibge.gov.br/).

The ANS system contains information about the private health system in Brazil. We extracted tables with the annual female population having private health insurance, aged from 50 to 69 years, in all the cities of the state of São Paulo in the years from 2017 to 2020 (http://www.ans.gov.br/anstabnet/index.htm).

The target women population, female users of the SUS, aged between 50 and 69 years, was obtained by subtracting the users of the private health system from the estimated total population in the same age interval.

The state of São Paulo has 17 Regional Health Departments (RHDs). The monthly screening mammograms per 1,000 target women and BIRADS 4 or BIRADS 5 mammograms per 100,000 target women were grouped by RHD.

Statistical analysis

We carried out the statistical analysis using the software R version 4.1.1 (2021-08-10). There were no missing data. We used the chi-square test to compare the changes in insured and public system users from 2017 to 2020, time-series graphical analyses to evaluate the changes in the monthly rate of screening mammograms, and a heatmap to evaluate the impact of the COVID-19 pandemic across the RHDs.

RESULTS

Table 1 summarizes the target population for breast cancer screening in the state of São Paulo from 2017 to 2020. The estimated women population, aged from 50 to 69 years, dependent on the public health system was 64% of the total in 2017 and increased to 66% in 2020 (X-squared = 6,183.2, df = 3, p < 0.0001).

Table 1. Distribution of women population aged between 50 and 69 years in the São Paulo state from 2017 to 2020.

Year	Private system	Public system	Total
2017	1,737,588	3,053,427	4,791,015
2018	1,737,196	3,171,960	4,909,156
2019	1,739,061	3,283,524	5,022,585
2020	1,745,572	3,386,350	5,131,922

The mean monthly screening mammograms per 1,000 target women was 12.5 in 2017, 13.3 in 2018, 14.8 in 2019, and 9.25 in 2020. Figure 1A shows the time-series representation of the monthly rate throughout this period. April 2020 (3.4/1,000) and May 2020 (3.1/1,000) had the lowest rates.

Figure 1B shows the time series for high-risk mammograms per 100,000 target women. The monthly mean of BIRADS 4 or BIRADS 5 per 100,000 women was 8.1 in 2017, 11.2 in 2018, 12.8 in 2019, and 9.1 in 2020. In April 2020, the rate was 4.3/100,000, and in May 2020, the rate was 4.8/100,000.

Figure 2 shows the monthly distribution of screening mammograms per 1,000 women aged between 50 and 69 years across the 17 departments of health in the state of São Paulo from 2017 to 2020. Mammographic coverage was heterogeneous, but there was a substantial decrease in all health departments in April and May 2020.

DISCUSSION

We observed a substantial reduction in the rates of screening mammograms and results of high-risk mammogram in the state of Sao Paulo with the onset of COVID-19 pandemic. The decrease in the rates occurred across the entire state. The rates slowly increased; however, there was no compensation for the initial decrease. This behavior may result in an increase in the rate of advanced tumors in the following months.

The strength of our study was the large number of mammograms analyzed in a well-defined scenario. As we used population-based datasets, the main limitations were selection bias and the impossibility of analyzing non-reported confounding variables.

In Brazil, breast cancer screening is opportunistic. Several authors discuss the differences between organized and opportunistic breast cancer screening programs. Organized screening programs have higher attendance and rate of detection of in situ breast cancer screening[11,12]. There are several barriers

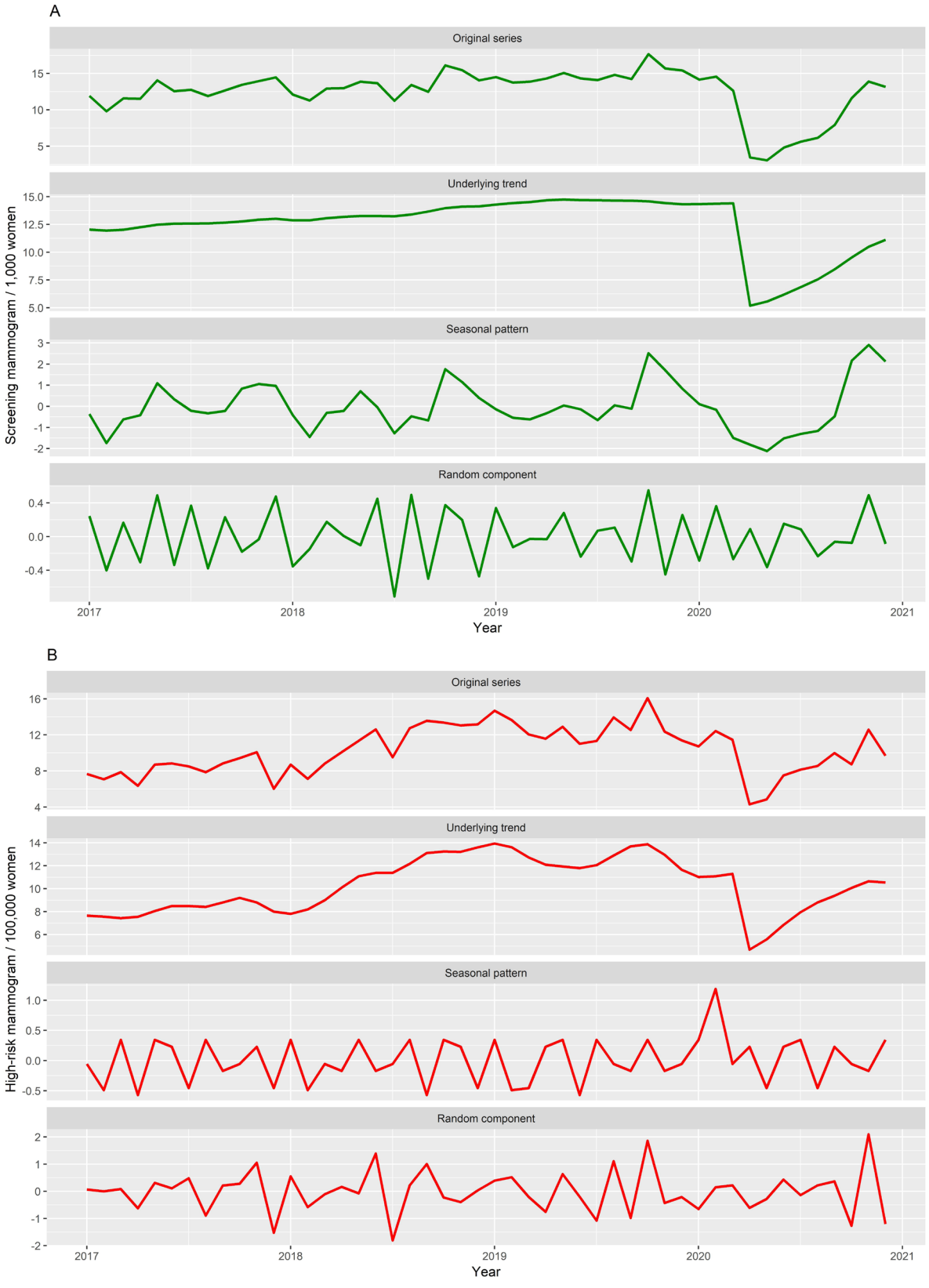

Figure 1. Time-series analysis of the monthly rate of screening mammograms (A) and high-risk mammograms (B) in the state of São Paulo from 2017 to 2020, showing a substantial decrease in April and May 2020.

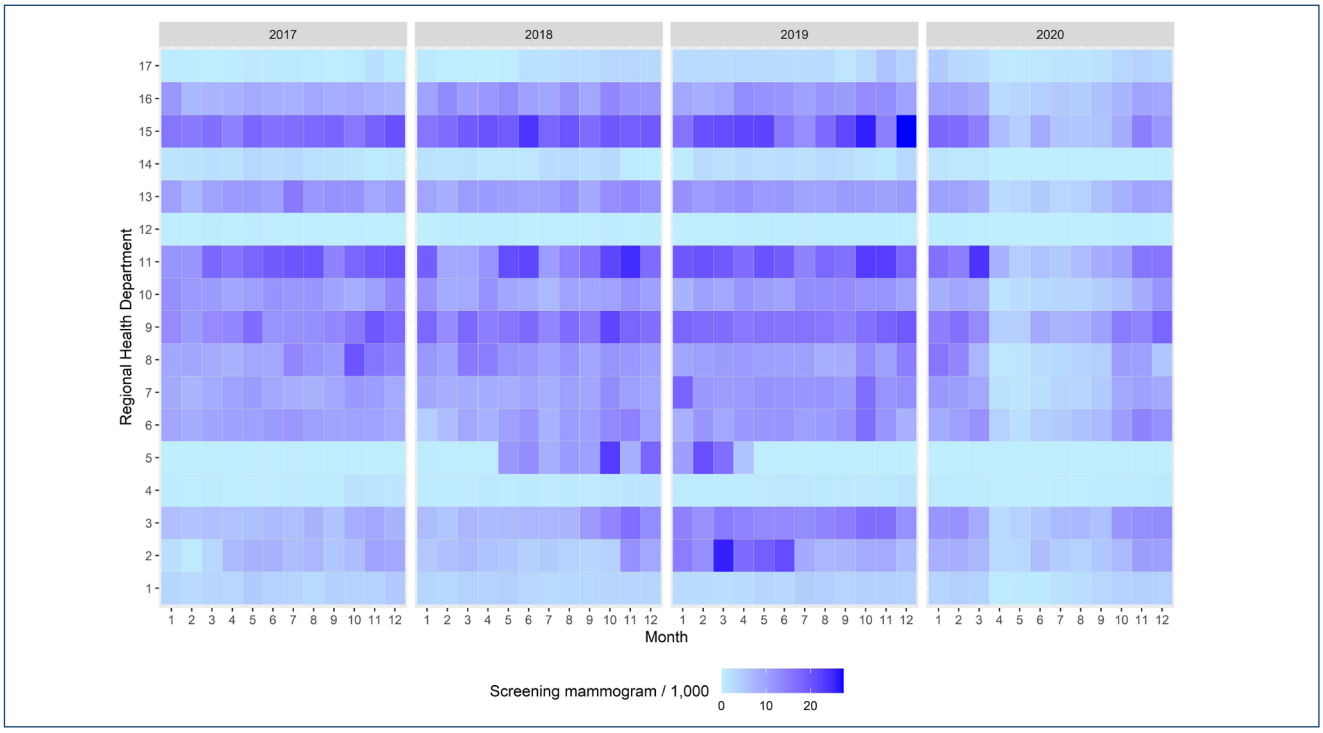

Figure 2. Heatmap of the monthly screening mammograms in the São Paulo state from 2017 to 2020.

to access to mammographic services in developing countries, such as travel distance, educational, financial, and social inequalities[13,14]. Also, in an opportunistic screening scenario, patient behavior is determinant for mammographic screening coverage. Many women, especially those assisted by the public health system, receive a mammography recommendation after visiting a physician and trust their doctors know the best time to request screening[10,15,16]. Health assistance disruptors, such as those from COVID-19 pandemic, make the weakness of an opportunistic breast cancer screening more evident. Even for a short period, the reduction in screening mammograms can reduce breast cancer diagnosis in the early stages and thus increase mortality[8,9].

We also noted a reduction in screening mammograms with BIRADS 4 or 5 results. This fact, associated with the screening mammograms returning only to pre-pandemic levels, suggests that a more significant number of mammograms with high-risk findings may appear in the future, with the potential to overload the public health system with demands of diagnosis procedures, such as biopsies[17]. Our results are similar to data previously reported in Southern Taiwan and Northern Italy[18,19]. Millions of breast cancer cases are diagnosed through breast screening in the world every year, and the reduction of screening may also increase mortality[7,20]. Some authors warn for a potential increase in advanced-stage breast cancers and mortality in the next decade related to the time of screening interruption[9,21].

New waves of COVID-19 may occur in the following months. Moreover, disrupters of health assistance may happen in the future by other causes. Health systems must be prepared to maintain screening programs in such a situation and avoid increasing advanced-stage breast cancer and mortality.

In conclusion, we reported a substantial reduction in mammographic screening related to the COVID-19 pandemic, which happened in all regional health departments in the state of São Paulo. However, the volume of mammograms is returning to pre-pandemic levels, but it is not enough to compensate for the disruption. We may have an increase in advanced-stage breast cancer diagnosis and even mortality in the following years.

AUTHORS' CONTRIBUTIONS

NJWMJ: Data curation, Formal Analysis, Investigation, Writing – review & editing. **VCM:** Validation, Writing – review & editing. **LBBGM:** Data curation, Validation, Writing – review & editing. **FFP:** Data curation, Validation, Writing – review & editing. **FJCR:** Conceptualization, Formal Analysis, Funding acquisition, Methodology, Supervision, Writing – original draft.

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ORIGINAL ARTICLE

AOP

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"Zooming" in strategies and outcomes for trauma cases with Injury Severity Score (ISS) ≥16: promise or passé?

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SUMMARY

OBJECTIVE: Rescuing severe trauma cases is extremely demanding. The present study purposed to analyze the efficiency of trauma management at Emergency Centre, University Clinical Centre of Serbia, Belgrade, included outcome within 28 days.

METHODS: This retrospective study involved 131 intensive care unit trauma cases with total Injury Severity Score ≥16, in terms of administrating the two strategies: (i) definitive surgical repair and (ii) damage control laparotomy.

RESULTS: The damage control laparotomy cases revealed statistically higher Injury Severity Score and APACHE II scores, significant brain dysfunction, and hemorrhagic shock on arrival (p<0.001). In addition, the damage control laparotomy had a higher rate of respiratory complications, multiple organ deficiency syndrome, and surgical wound complications (p=0.017, <0.001, and 0.004, respectively), with more days on mechanical ventilation (p=0.003). Overall mortality was 29.8%. Although higher early mortality within ≤24 h in the damage control laparotomy (p=0.021) had been observed, no difference between groups (p=0.172) after the 4th day of hospitalization was detected.

CONCLUSIONS: Trauma patients have a high mortality rate in the 1st hours after the incident. Compelling evidence linking host and pathogen factors, such as mitochondrial apoptosis pathways, appears to correlate with loss of organ dysfunction, both cytopathologically and histopathologically. Adequate selection of patients necessitating damage control laparotomy, allowed by the World Society of Emergency Surgery, abdominopelvic trauma classifications, and improvements in resuscitation, may improve the results of severe trauma treatment.

KEYWORDS: Wounds and injuries. Emergencies. Pathology. Mortality.

INTRODUCTION

Severe abdominal trauma associated with injuries could have been accompanied by fatal complications, and its management remains a great challenge. In a severe trauma patient, the biggest issues for the surgeon are choosing the most appropriate surgical approach and rapid controlling of injury. Severely traumatized patients could develop swiftly life-threatening traumatic coagulopathy induced by massive bleeding, hemodynamic shock, deregulation of the coagulation cascade, and activation of anticoagulant and fibrinolytic pathways. Definitive surgical repair (DSR) is a traditional approach dealing with convenient injuries during initial emergency laparotomies. However, damage control laparotomy (DCL) has been established in order to minimize the pathophysiological impact of the relevant lethal triad[1,2]. The present study analyzes the effectiveness of two kinds of surgical approaches, DCL vs. DSR, in severely injured patients who had been treated multidisciplinary at the Emergency Centre, University Clinical Centre of Serbia, Belgrade, during a 1-year interval, considering the outcomes within the 28-day in-hospital mortality.

METHODS

A total of 131 adult polytrauma cases (Injury Severity Score [ISS] ≥16), who had undergone the emergency laparotomy, due to the abdominal injuries grades III–V according to the American Association for the Surgery of Trauma (AAST), had

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been involved in the present retrospective study[3]. Dead on arrival and patients treated by nonoperative management (NOM) had been excluded from the study by administrating the cases with the two surgical approaches, DCL vs. DSR, for the therapeutic purposes[4]. To this end, DSR had been opted for a total of 109 (83.2%) patients, through the midline incision abdominal exploration, followed by a hemostatic control and definitive injury repair, such as the simple suture for the intestinal injury, organ resection, selective vascular ligation, and debridement of the extensive devitalized tissue, whereas DCL had been applied in 22 (16.8%) patients with an indication of life-saving bleeding control in the hemodynamically unstable cases who had not responded to the initial resuscitation due to the complex liver injuries, pelvic fracture, and physiological derangement as the metabolic acidosis (lactate<5 mmol/L, pH<7.2, base deficit>14), hypothermia (temperature<34°C), and coagulopathy (INR>1.5 with PT and PTT>2 folds). The first stage of the DCL was middle line laparotomy, followed by a rapid control of life-threatening hemorrhage and source control for a hollow viscus perforation. In case of liver trauma, blood vessels were suture/ligated before the packing and the Pringle maneuver had been used temporarily for providing the inflow vascular control purposes. For the perihepatic packing procedure, we used approximately 4–6 sterile abdominal swabs, which were never placed directly onto the liver laceration or hematoma. During the explorative laparotomy in cases with the concomitant unstable complex pelvic fracture and progressive retroperitoneal hematoma, we indicated the transabdominal pelvic packing. Arterial and major venous injuries within the internal iliac distribution had been controlled by vascular repair or ligation while the pelvic tamponade had been used to control venous hemorrhage from the presacral plexus and prevesical veins, followed by an orthopedic external pelvic stabilization. As the second stage had comprised the multidisciplinary treatment in an intensive care unit (ICU) to stabilize the physiological status of the cases in the next 48 h, the third stage in DCL had been a planned re-laparotomy, including removal of the abdominal packing and definitive surgical reconstruction. All the cases had been followed up for 28 days. Major complications were defined as Clavien-Dindo grade≥3: diffuse peritonitis, traumatic coagulopathy, ventilator-associated pneumonia (VAP), sepsis, and multiple organ deficiency syndrome (MODS)[5-8], while early mortality as a death occurred within the first 24 h and the late one occurred after 96 h.

Statistical analyses

Depending on the type of variables and the normality of the distribution, results were presented as frequency (percent), median (range), and mean±standard deviation. The statistical hypotheses were tested using (i) the Student's t-test, (ii) the Mann-Whitney U test, (iii) the chi-square test, and (iv) the Fisher's exact test. A statistical hypothesis was analyzed at the level of significance of 0.05, while the statistical data analyses were performed using IBM SPSS Statistics 22.0 (IBM Corporation, Armonk, NY, USA).

RESULTS

No significant difference was recognized in terms of sex, age, comorbidity, and mechanism of injury (p>0.05, Table 1). The leading cause of the blunt trauma occurred due to road

Table 1. Demographic and initial clinical presentation on admission for the damage control laparotomy vs. definitive surgical repair.

Characteristics	DCL n (%) 22 (16.8)	DSR n (%) 109 (83.2)	p
Sex			
Male	20 (90.9)	94 (86.2)	0.736
Female	2 (9.1)	15 (13.8)	
Age (years)[a]	42.23±20.84	40.90±16.64	0.781
Comorbidity ≥2	7 (31.8)	20 (18.3)	0.160
Blunt trauma	20 (90.9)	80 (73.4)	0.100
SBP<90	18 (81.8)	34 (32.2)	<0.001
Hemorrhagic shock	21 (95.5)	23 (21.1)	<0.001
GCS≤12[b]	14 (63.6)	26 (23.9)	<0.001
ISS[a]	40.05±11.01	29.75±9.46	<0.001
APACHE II[a]	25.50±3.39	21.06±5.15	<0.001
Retroperitoneal hematoma (RPH)	9 (40.9)	30 (27.5)	0.050
Stomach AAST ≥3	4 (18.2)	6 (5.5)	0.064
Small intestine AAST ≥3	4 (18.2)	18 (16.5)	0.764
Colon AAST ≥3	7 (31.8)	20 (18.3)	0.160
Pancreas AAST ≥3	2 (9.1)	6 (5.5)	0.621
Kidney AAST ≥3	3 (13.6)	6 (5.5)	0.175
Thorax injury AIS≥3	19 (86.4)	58 (53.2)	0.004
Orthopedic injury AIS≥3	17 (77.3)	35 (32.1)	<0.001
Spinal injury AIS≥3	4 (18.2)	16 (14.7)	0.746
Head injury AIS≥3	13 (59.1)	26 (23.9)	0.001
Maxillofacial injury AIS≥3	4 (18.2)	16 (14.7)	0.746
Vascular injury AIS≥3	3 (13.6)	12 (11.0)	0.717

SBP: systolic blood pressure; GCS: Glasgow Coma Scale; ISS: Injury Severity Score; APACHE II: Acute Physiology and Chronic Health Evaluation II score; DCL: damage control laparotomy; DSR: definitive surgical repair. [a]Mean ±SD. [b]GCS score ≤12 utilized for defined moderate to severe brain injury.

traffic accidents (p>0.05). The DCL cases revealed significant hypotension on arrival, hemorrhage shock, and brain injury (p<0.001 for all, Table 1). The DCL vs. DSR had significantly higher ISS (40.05±11.01 vs. 29.75±9.46) and APACHE score (25.50±3.39 vs. 21.06±5.15) (p<0.001, Table 1). In addition, the DCL exhibited significantly more extra-abdominal injuries: (i) the thorax (86.4 vs. 53.2%), (ii) orthopedic (77.3 vs. 32.1%), and (iii) the head (59.1% vs. 23.9%) (p=0.004, <0.001, and 0.001, respectively). A significant rate of severe liver and pelvis injury, according to WSES and AAST classification, was revealed in the DCL (Table 2), whereas the spleen was the most frequently injured abdominal solid organ in both groups (36.4% vs. 41.3%, p>0.05). We performed the liver packing procedure in half of the cases with severe liver injury, while the implementation of transabdominal pelvic packing with the external fixation of the pelvis was done in the other half in DCL due to severe pelvic trauma. The definitive surgery for the liver trauma was performed by the liver resection in 5 (4.6%) cases, while the parenchyma suture was performed in 23 (21.1%) cases. A statistically more hollow viscus suture was recognized in the DCL (p=0.001, Table 3). Herein, no significant numerical difference was found (p>0.05) while performing the splenectomy (22.7 vs. 41.3%), distal pancreatectomy (4.5 vs. 4.6%), adrenalectomy (4.5 vs. 1.8%), nephrectomy (4.5 vs. 3.7%), repair of bladder (18.2 vs. 8.3%), and hollow viscus resection (13.6% vs. 20.2%) . In addition, significantly more patients in the DCL had developed the traumatic coagulopathy (p=0.006), who had received more red blood cells (RBC) units of transfusion within the first 24 h (p=0.002), including massive blood transfusion (MBT) protocol (p=0.001, Table 3). The sepsis had developed in 28 (21.4%) cases in total without any significance (p>0.05). The DCL had been recognized as possessing significant respiratory complications, such as VAP and pleural effusion (p=0.017). Of 32 (24.4%) cases, VAP was recorded without a significance (31.8% vs. 21.9%, p>0.05) and 39 (29.8%) cases revealed the pleural effusion followed by the basal lung segment atelectasis, without difference between the groups (45.5 vs. 26.6%, p>0.05). The DCL cases had a significantly higher rate of MODS (p<0.001) and significantly more patients had surgical wound complications (p=0.004) (Table 3). Notably, two (1.5%) patients had intestinal fistula, four (3.1%) had pancreatic fistula, and six (4.6%) had bile leakage, without significant difference (p>0.05). The postoperative course had not revealed a statistical difference between ICU stay and overall hospital stay, though the DCL had possessed significantly more days on the mechanical ventilation (p=0.003). Overall mortality in the present study had been detected as 29.8%. Analyzing outcomes in time interval had revealed significantly higher mortality within ≤24 h in the DCL cases involved in the present study (p=0.021).

DISCUSSION

It is a critical emergency surgery in which clinicians stay vigilant of DCL for unstable trauma patients as a life-saving surgical approach of rapid hemostatic and the relevant source control for gastrointestinal injury with temporary wound closure[9-11]. To this end, DSR, in terms of being a kind of time-consuming procedure, is not usually recommended in trauma cases with critical physiological status, despite excellent surgical techniques[12]. The rate of cases treated by the DCL approach in the present study was 16.8%, compared to 6–18% in the other studies[13]. However, Hommes et al.[12] reported treating

Table 2. World Society of Emergency Surgery and American Association for the Surgery of Trauma classification for liver, spleen, and pelvis trauma[3,9,10,11].

		WSES grade		p	AAST grade		p
		Moderate	Severe		Moderate	Severe	
		II+III n (%)	IV n (%)		III n (%)	IV+V n (%)	
Liver	DSR	29 (82.9)	0 (0.0)	<0.001	24 (100.0)	5 (31.2)	<0.001
	DCL	6 (17.1)	5 (100.0)		0 (0.0)	11 (68.8)	
Spleen	DSR	34 (91.9)	11 (84.6)	0.594	12 (92.3)	33 (89.2)	1.000
	DCL	3 (8.1)	2 (15.4)		1 (7.7)	4 (10.8)	
Pelvis	DSR	22 (84.6)	0 (0.0)	<0.001	15 (100.0)	7 (38.9)	<0.001
	DCL	4 (15.4)	7 (100)		0 (0.0)	11 (61.1)	

WSES: The World Society of Emergency Surgery; AAST: The American Association for the Surgery of Trauma; DCL: damage control laparotomy; DSR: definitive surgical repair.

Table 3. The surgical procedures, ICU clinical course, and outcomes in the damage control laparotomy vs. definitive surgical repair.

Characteristics	DCL n (%) 22 (16.8)	DSR n (%) 109 (83.2)	p
Splenectomy	5 (22.7)	45 (41.3)	0.102
Liver resection	1 (4.5)	5 (4.6)	0.993
Liver suture	2 (9.1)	22 (20.2)	0.364
Hollow viscus suture	12 (54.5)	22 (20.2)	0.001
Maxillofacial surgery	3 (13.6)	12 (11.0)	0.717
Neurosurgical interventions	3 (13.6)	15 (13.8)	1.000
Orthopedic surgery	13 (59.1)	19 (17.4)	<0.001
Spinal surgery	1 (4.5)	6 (5.5)	1.000
Surgical tracheostomy$^{\alpha}$	8 (36.4)	16 (14.7)	0.030
Surgical gastrostomy$^{\alpha}$	8 (36.4)	14 (12.8)	0.012
Chest tube	14 (63.6)	42 (38.5)	0.030
Emergency thoracotomy	1 (4.5)	3 (2.8)	0.525
Emergency vascular procedures	2 (9.1)	5 (4.6)	0.334
MBT[a]	14 (63.6)	28 (25.7)	0.001
RBC units ≥24 h[a]	10 (2–20)	5 (2–26)	0.002
Traumatic coagulopathy	19 (86.4)	9 (8.3)	<0.001
Sepsis	8 (36.4)	20 (18.3)	0.085
Respiratory complications	17 (77.3)	54 (49.5)	0.017
MODS	12 (54.5)	19 (17.4)	<0.001
Surgical wound complications	7 (31.8)	8 (7.3)	0.004
Abdominal complications	3 (13.6)	9 (8.2)	0.693
Re-laparotomy	0 (0.0)	5 (4.6)	0.589
MV $^{day\ 1}$	5 (0–19)	1 (0–20)	0.003
ICU $^{day\ 1}$	7 (1–24)	4 (0–25)	0.124
Hospital length of stay $^{day\ 1}$	11 (1–28)	10 (1–28)	0.535
Early mortality ≤24 h	6 (27.3)	9 (8.3)	0.021
Late mortality ≥96 h	3 (13.6)	14 (12.8)	0.172
Mortality 24–96 h	0 (0)	7 (6.4)	0.359
Mortality	9 (40.9)	30 (27.5)	0.210

$^{\alpha}$Tracheostomy was indicated when MV was prolonged (≥10 days) after the first spontaneous breathing trial. $^{\beta}$Gastrostomy for enteral feeding indicated during prolongated MV (≥10 days). RBC: red blood cells; MBT: massive blood transfusion; ICU: intensive care unit; MODS: multiple organ dysfunction syndrome; MV: mechanical ventilation. [a]Med (min–max).

31% of patients using the DCL approach for liver trauma on severe abdominal injuries in the right upper quadrant. We also reported complex hepatic trauma management in which the liver packing had been performed at the rate of 48.8%[14]. According to these data, the DCL approach becomes more frequent in the more selected cases of hepatic injury. In the present study, the DCL exhibited more orthopedic, thoracic, and head injuries with the additional extrabdominal surgical procedures.

Uncontrolled bleeding is considered a dominant cause of early trauma-related death in severe abdominal trauma[1,2,15,16]. Of note, the classification of organ injuries should be related not only to the topography but also to associated physiological derangement[3,9,10,11], which might lead to more tailored management. Severe pelvic trauma with massive hemorrhage strongly contributes to a high mortality rate in polytrauma patients[17]. Complex treatment of pelvic trauma includes procedures to achieve hemodynamic stability and stabilization of the pelvic ring[17,18]. We performed the DCL transabdominal pelvic packing in the exsanguinated patients with associated complex pelvic trauma. According to the European guideline on management of major bleeding and coagulopathy following trauma, severely injured patients who presented with deep hemorrhagic shock, signs of bleeding, and coagulopathy should undergo DCL (Strong Recommendation [Grade 1B])[2].

Severe trauma results in a strong inflammatory response and life-threatening complications. VAP in the ICU can be expected in critically injured cases who are on mechanical ventilation (MV)[19], for more than 2 days, with an incidence ranging from 5% to 40% and a mortality rate of 10%[20]. The complex pathophysiology of MODS after trauma includes multifactorial pathologies such as initial exsanguination, MBT, and systemic inflammatory response, of which the most crucial is the severity of injury[21]. Multifactorial impairment of physiological status is the main reason for death in patients with severe trauma[6-13]. The severity of mitochondrial pathology was reported to correlate with sepsis-induced cell and organ failure, both cytopathologically and histopathologically[22]. Harvin et al.[23] stated that mortality following penetrating abdominal trauma was 10%, while it was much higher than 40% for blunt trauma. In the present study, the overall 28-day mortality was detected in 29.8%. The analysis of mortality concerning the period postulated that more patients die within the first 4 days. The deadliest period for the DCL was the first 24 h, with a significant difference in mortality for that interval between groups (27.3% vs. 8.3%). After 96 h, the distribution of mortality rates had become similar for both. Despite the high mortality rate, in the DCL, almost 60% of the cases survived the potentially fatal injuries, estimating the damage control in a multidisciplinary approach for hemostasis, and future improvements of initial resuscitation will be able to achieve rescuing the most severely traumatized patients.

CONCLUSIONS

In emergency evaluation, trauma cases have a high mortality rate in the 1st hours after the incident. The prevention of early deaths and improvements in resuscitation can increase the chances of survival. Adequate selection of the cases requiring DCL procedure might improve the outcomes of therapeutic approaches for severe trauma cases. Furthermore, preliminary evidence currently indicates that to resolve these issues, opting for the optimal approach, such as DCL and DSR, could also play an important role in trauma cases, at least for significant comorbidities. For this purpose, additional studies are needed to address which specific emergency surgery treatment modality is optimal for this controversial issue.

AUTHORS' CONTRIBUTIONS

KD: Conceptualization, Data curation, Formal Analysis, Investigation, Methodology, Project administration, Resources, Software, Validation, Visualization, Writing-original draft. **ZL:** Conceptualization, Data curation, Formal Analysis, Investigation, Methodology, Project administration, Resources, Software, Validation, Visualization, Writing-original draft. **FC:** Conceptualization, Data curation, Formal Analysis, Investigation, Methodology, Project administration, Resources, Software, Validation, Visualization, Writing-original draft. **PG:** Conceptualization, Data curation, Formal Analysis, Investigation, Methodology, Project administration, Resources, Software, Validation, Visualization, Writing-original draft. **DM:** Conceptualization, Data curation, Formal Analysis, Investigation, Methodology, Project administration, Resources, Software, Validation, Visualization, Writing-original draft. **ZB:** Conceptualization, Data curation, Formal Analysis, Investigation, Methodology, Project administration, Resources, Software, Validation, Visualization, Writing-original draft. **PD:** Conceptualization, Data curation, Formal Analysis, Investigation, Methodology, Project administration, Resources, Software, Validation, Visualization, Writing-original draft. **DS:** Investigation, Software, Supervision, Writing – review & editing. **IS:** Investigation, Methodology, Software, Supervision, Writing – review & editing.

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ORIGINAL ARTICLE

AOP

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Do heart rate variability indices present potential to predict late postmenopausal? A retrospective study

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SUMMARY

OBJECTIVES: This study aimed to compare heart rate variability indices in early and late postmenopausal women and assess their correlation and prognostic value to predict late postmenopausal.

METHODS: An observational and retrospective study was performed with the medical records of patients from Hospital das Clínicas, Faculdade de Medicina da Universidade de Sao Paulo between 2018 and 2019. We selected medical records of women with menopause, over 40 years old, which were divided into two groups, according to postmenopausal time, i.e., early and late postmenopausal.

RESULTS: We analyzed data from 123 women (55 in the early and 68 in the late postmenopausal group). RRtri (triangular index) was lower in the late postmenopausal group (8.68 vs. 7.15, p=0.040). There was a significant weak negative correlation in SDNN, RRtri, and SD2 and postmenopausal time. RRtri presented the potential to predict late postmenopausal.

CONCLUSION: The increase in postmenopausal time decreases global heart rate variability indices. The geometric index RRtri was significantly lower in late postmenopausal women and presented the potential to predict late postmenopausal.

KEYWORDS: Autonomic nervous system. Climacteric. Postmenopause. Heart rate determination. Gynecology. ROC curve.

INTRODUCTION

The postmenopausal period can be divided into early and late postmenopausal. Early postmenopausal is defined as the period of up to 6 years after menopause, in which follicle-stimulating hormone (FSH) levels remain high; there is a progressive reduction in estradiol and greater acceleration of bone loss. The late phase starts from the 6th year after menopause and goes until senectivity[1,2]. In general, it is characterized by menopausal symptoms and changes in different systems, including cardiac autonomic modulation[2,3].

Apparently, hypoestrogenism changes the autonomic control of heart rate (HR), inducing alterations in sympathetic and vagal regulation. Previous studies[1-4] have observed that cardiac parasympathetic function is reduced in postmenopausal women, due to aging and hormone level, when compared with pre- or transition to menopause. Other studies indicate that certain interventions, such as physical exercise, could increase the heart rate variability (HRV)[4], the parasympathetic modulation[5], and the system complexity[3] in postmenopausal women.

Indeed, HRV as a measure of the autonomic nervous system function has been used in several conditions, including in menopause[1-3,5]. However, these studies do not divide their population into early and postmenopausal groups. In general, the menopausal group consists of women with different years without a menstrual cycle, and the authors generalized their conclusions, regardless of the possible influence that the postmenopause time could have on cardiac regulation.

In this sense, considering that previous studies[6-9] show that some linear and nonlinear HRV indices have good diagnostic accuracy in certain populations, and with the intention of distinguishing the postmenopause stages, our objectives were to compare HRV indices in early and late postmenopausal women and to assess their correlation and prognostic value to predict late postmenopausal.

METHODS

Study design and ethical considerations

This observational and retrospective study was performed using the medical records of patients seen at Climacteric Sector,

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Gynecology Division, Hospital das Clínicas, Faculdade de Medicina da Universidade de Sao Paulo (FMUSP) between 2018 and 2019.

This study was approved by the Research Ethics Committee of the same institution (number 1.977.216). The written informed consent was obtained from the participants, and their anonymity was guaranteed, according to the Helsinki Declaration and the 466/2012 resolution of the Brazilian National Health Council.

Population and eligibility criteria

As inclusion criteria, we selected medical records of women with a clinical (absence of menstruation for a period longer than 12 months) or laboratory (FSH > 30 IU/L) diagnosis of postmenopause, composing a non-probabilistic sampling of convenience.

Exclusion criteria were primary or premature ovarian failure; active gynecological neoplasia; thromboembolic disorders; active parathyroid and thyroid endocrine disorders without treatment; malabsorption syndrome; endometrial thickening (suspected endometrial cancer); abuse of alcohol or illicit drugs and psychiatric illness in treatment, use of beta-blockers, acute cardiovascular disease, and arrhythmias in pregnancy; morbid obesity (body mass index [BMI] ≥40 kg/m^2); insulin-dependent diabetes mellitus; and current diagnosis of neoplasms and/or under treatment. All these information were obtained from medical records. For this reason, we also excluded records with incomplete information.

Then we divided them into two groups, according to postmenopause time[2], i.e., early (≤6 years) and late (>6 years) postmenopausal. From this, we have excluded those HRV data with an error greater than 5% in the RR intervals (RRis). Both the sample composition process and the analyses were carried out by an independent researcher. Figure 1 shows the sample selection process.

Sociodemographic and clinical characteristics

For characterization of volunteers, we recollected information about age (years), postmenopausal time (years), last menstruation age (years), menarche age (years), reproductive cycle time (years), BMI, race/ethnicity (i.e., white, mixed race, and black), marital status (stable union and unstable union), exercise (yes or no), and smoking (yes or no).

Heart rate variability analysis

For the HRV analysis, we have used the methodologies proposed by Catai et al.[10] and Vanderlei et al.[11]. Initially, the RRis were recorded by a validated HR receiver (acquisition rate 1000 Hz) validated equipment for HR capturing beat by beat, during 20 min in rest supine, individually in a quiet environment with the minimum circulation of people[10]. To standardize circadian influences, all HR records were performed at the same time of the day (8:00 to 12:00 a.m.) in a room with a temperature between 22°C and 25°C[10,11].

The data series was first digitally filtered, in which only series with more than 95% sinus rhythm beats were included. Then, it was manually complemented, and the visual inspection of the time series on the computer showed the absence of artifacts. Finally, 1000 consecutive RRis were selected for data analysis[10,11]. We used Kubios HRV Analysis software (v.1.1, Biomedical Signal Analysis Group, Department of Applied Physics, University of Kuopio, Finland) for the HRV analysis in linear (time and frequency domains) and nonlinear methods[10,11].

Linear and nonlinear heart rate variability indices

We have performed a linear (time and frequency domains) and nonlinear analysis of HRV. In the time domain, we have analyzed statistics and geometric indices. The statistics indices were as follows: MEANRR (median of RRis/ms), SDNN (mean standard deviation of all normal RRis/ms), and RMSSD (square root of the mean of squared differences between successive beat intervals/ms)[10,11].

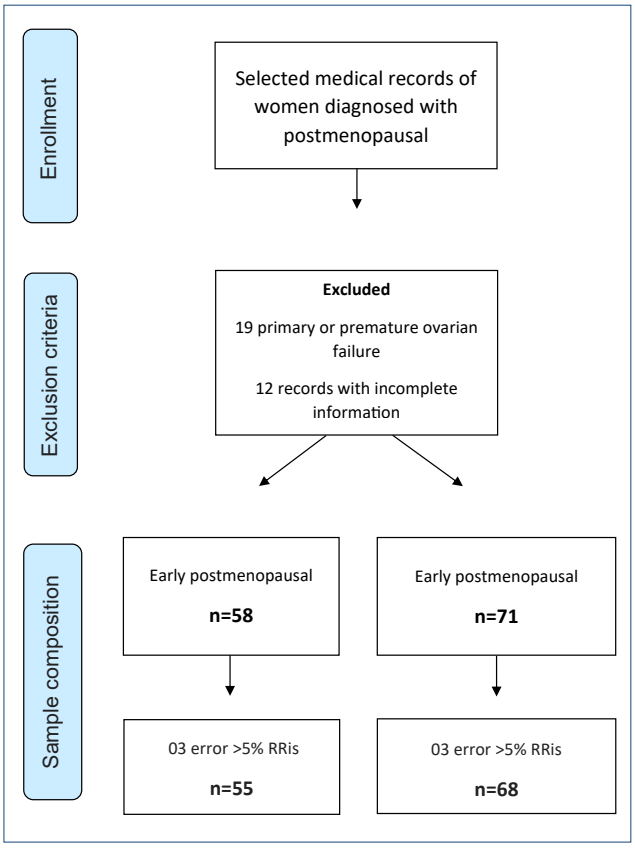

Figure 1. Sample selection process.

The geometric indices were as follows: SD1 (standard deviation of the instantaneous variability in continuous RRis, determined by the width of the ellipse formed by the Poincare plot), SD2 (standard deviation of long-term continuous RRis, which determines the length of the plot), SD1/SD2 (ratio between short and long variations of the intervals), RRtri [triangular index, calculated from the construction of the histogram density of normal RRis and obtained by integral division of the histogram (RRis total number) by the maximum density distribution (RRi modal frequency), obtained with a sampling frequency of 128 Hz] and TINN (RRi triangular interpolation, distribution baseline width measured as a triangular base, approximating the distribution of all RRi)[4,11].

In the frequency domain, we studied the spectral indices obtained by Fourier Transform mathematical algorithm. The indices were obtained from the frequency bands, in which high-frequency (HF) fluctuations ranging from 0.15 to 0.4 Hz, low-frequency (LF) fluctuations ranging from 0.04 to 0.15 Hz, and LF/HF ratio. These indices were presented in normalized units (nu) and ms[2,10,11].

In the nonlinear methods, we have analyzed the fractals proprieties and entropy for evaluating the system complexity. The fractal analysis was performed by indexes ALPHA1 (short-term fractal scaling exponent, which corresponds to a period of 4–11 beats), ALPHA2 (long-term fractal scaling exponent that is longer than 11 beats), and ALPHA1/ALPHA2 (short-term fractal scaling exponent/long-term fractal scaling exponent ratio). Entropy is an approach used to quantify the regularity (complexity) of the RRi series fluctuations. As some entropy measurements require relatively short recordings, we have evaluated the sample entropy (SAMPEN), which needs <200 points[11].

Statistical analyses

Data normality was initially determined by the Shapiro-Wilk test. To describe the variables, the median and the standard deviation values were used for quantitative variables, and absolute and relative frequency for qualitative variables. Comparisons between early postmenopausal and late postmenopausal women were performed using the Mann-Whitney test and chi-square test. In addition, Spearman's correlation test was used to compare the HRV and time since menopause.

The definition of the cutoff points for the HRV indices was obtained by the receiver-operating characteristic (ROC) curve. In addition, sensitivity, specificity, positive predictive values, and negative predictive values for the occurrence of the late postmenopausal phase were obtained. The cutoff point was determined by the Youden index[12]. The area under the curve was considered significant when values ≥0.650 were obtained[8]. For all analyses, a 95% confidence level was used. The program used was Stata version 13.0.

RESULTS

We analyzed data from 160 women, of which 37 were excluded. Sociodemographic and clinical profiles and comparison between HRV indices of the women evaluated are shown in Table 1.

Table 2 presents the correlation between HRV indices and postmenopausal time. There was a significant weak negative correlation in SDNN, RRtri, and SD2.

Table 3 shows sensitivity, specificity, ROC curve, and positive and negative predictive values of HRV indices to predict late postmenopausal. The geometric index RRtri presented the potential to predict late postmenopausal.

DISCUSSION

The main results of this study are that there was a significant weak negative correlation in global HRV indices and postmenopausal time, and the RRtri presented potential to predict late postmenopausal. In addition, except for the RRtri, there were no differences in autonomic modulation between women with early or late postmenopause.

Regarding sociodemographic and clinical characteristics, because of groups characteristics, differences were already expected. Also, we have observed no differences in their BMI, smoking, and exercise habits, which are factors that can influence cardiac autonomic modulation[3,13]. Overweight has been reported in other studies, and it is a comorbidity associated with menopausal status and increased cardiovascular risk in this population[3,13]. About exercises, many studies evaluated the effects of different physical exercise programs on autonomous regulation and found an increase in HRV[3-5], parasympathetic modulation[5], and system complexity[3] in postmenopausal women.

In the analysis of linear and nonlinear HRV indices, only RRtri was significantly lower in postmenopausal women, indicating reduced global HRV in this group. Interestingly, other global HRV indices (i.e., SDNN and SD2) also tended to decrease. As for the others, they were not different between groups. Most studies[1-3] on cardiac autonomic modulation in postmenopausal women do not divide the subjects by the postmenopausal time. In general, they carry out evaluations considering the period before and after menopause, and their results suggest decreased parasympathetic regulation of HR in the postmenopause in general. To the best of our knowledge, only one study[14] divided its sample according to the stages of

Table 1. Sociodemographic and clinical characteristics and heart rate variability indices of the postmenopausal women (Sao Paulo, Brazil).

Variables	Early (n=55)	Late (n=68)	p[a]
	Mean (SD)		
Age (years)	54.01 (3.69)	63.07 (7.28)	<0.001[a]
Age of last menstruation (years)	50.60 (3.73)	47.61 (4.45)	<0.001[a]
Postmenopausal time (years)	3.41 (1.68)	15.45 (7.24)	<0.001[a]
Menarche age (years)	12.92 (1.75)	12.52 (1.85)	0.136
Reproductive cycle time (years)	37.67 (4.29)	35.08 (5.11)	0.009[a]
BMI	27.67 (4.11)	27.19 (3.95)	0.566
	n (%)		p[b]
Race/ethnicity			
White	21 (39.62)	42 (62.69)	0.030[a]
Mixed race	24 (45.28)	21 (31.34)	
Black	8 (15.09)	4 (5.97)	
Marital status			
Stable union	25 (45.45)	35 (52.24)	0.904
Unstable union	30 (54.54)	33 (47.76)	
Exercise			
Yes	25 (54.55)	31 (53.73)	0.928
No	30 (45.45)	36 (46.27)	
Smoking			
Yes	8 (84.91)	5 (91.94)	0.235
No	45 (15.09)	57 (8.06)	
HRV indices	**Early (n=55)**	**Late (n=68)**	**p[c]**
	Mean (SD)		
MEANRR	880.70 (135.39)	909.68 (119.82)	0.153
SDNN	33.71 (26.07)	25.55 (10.17)	0.059
RMSSD	24.43 (13.81)	21.66 (10.08)	0.388
RRtri	8.68 (4.21)	7.15 (2.56)	0.040[c]
TINN	153.89 (118.14)	126.76 (51.18)	0.115
LF, ms²	378.34 (409.27)	254.80 (258.67)	0.230
LF, nu	59.26 (18.63)	58.24 (18.47)	0.678
HF, ms²	265.18 (270.44)	180.26 (181.15)	0.123
HF, nu	40.82 (18.31)	41.63 (18.44)	0.708
LF/HF	2.06 (1.59)	2.21 (2.28)	0.695
SD1	18.86 (14.38)	15.33 (7.14)	0.259
SD2	43.42 (36.77)	31.96 (14.32)	0.056
SD1/SD2	1.25 (0.86)	1.28 (0.80)	0.929
SAMPEN	1.58 (0.35)	1.62 (0.36)	0.407
ALFA1	1.08 (0.27)	1.04 (0.28)	0.389
ALFA2	0.62 (0.33)	0.59 (0.29)	0.963
ALFA1/ALFA2	2.13 (0.96)	2.03 (0.75)	0.530

[a]Mann-Whitney U test. [b]Chi-square test. [c]p<0.05, Mann-Whitney U test. SD: standard deviation; BMI: body mass index. SD: standard deviation; MEANRR: median of RR intervals/ms; SDNN: mean standard deviation of all normal RRis/ms; RMSSD: square root of the mean of squared differences between successive beat intervals/ms; HF: high frequency; LF: low frequency; LF/HF: low-frequency/high-frequency ratio; SD1: standard deviation of the RRis; SD2: standard deviation of long-term continuous RRis; SD1/SD2: ratio between short and long variations of the intervals; RRtri: triangular index; TINN: RRi triangular interpolation; SAMPEN: sample entropy; ALPHA1: short-term fractal scaling exponent; ALPHA2: long-term fractal scaling exponent; ALPHA1/ALPHA2: short-term fractal scaling exponent/long-term fractal scaling exponent ratio.

Table 2. Correlation between heart rate variability indices and postmenopausal time (Sao Paulo, Brazil).

Variables	Rho	p
MEANRR	0.037	0.678
SDNN	-0.193	0.032[a]
RMSSD	-0.093	0.303
RRtri	-0.188	0.036[a]
TINN	-0.087	0.335
LF, ms²	-0.121	0.181
LF, nu	-0.003	0.969
HF, ms²	-0.139	0.125
HF, nu	-0.0001	0.999
LF/HF	-0.001	0.987
SD1	-0.104	0.249
SD2	-0.198	0.027[a]
SD1 SD2	0.087	0.334
SAMPEN	0.050	0.580
ALFA1	-0.012	0.891
ALFA2	-0.035	0.697
ALFA1/ALFA2	0.058	0.518

Rho: Spearman's correlation test. [a]p<0.05. MEANRR: median of RR intervals/ms; SDNN: mean standard deviation of all normal RRis/ms; RMSSD: square root of the mean of squared differences between successive beat intervals/ms; HF: high frequency; LF: low frequency: LF/HF: low-frequency/high-frequency ratio; SD1: standard deviation of the RRis; SD2: standard deviation of long-term continuous RRis; SD1/SD2: ratio between short and long variations of the intervals; RRtri: triangular index; TINN: RRi triangular interpolation; SAMPEN: sample entropy; ALPHA1: short-term fractal scaling exponent; ALPHA2: long-term fractal scaling exponent; ALPHA1/ALPHA2: short-term fractal scaling exponent/long-term fractal scaling exponent ratio.

menopause, but the authors did not discuss the differences within the postmenopause group.

Regarding the correlation between HRV indices and groups, there was a significant weak negative correlation in SDNN, RRtri, and SD2. These indices express global HRV[10,11], which could indicate that with the increase in postmenopausal time there would be a reduction in the global variability. Global variability can be reduced mainly for two physiological mechanisms: (1) either decreased parasympathetic activity alone or both sympathetic and parasympathetic systems, and (2) or even by an increase in the sympathetic branch that leads to an imbalance in the autonomous function. In our study, we believe that the justification for these results may be the reduction in the parasympathetic system. Therefore, the discrimination of late menopause may be related to indexes that are influenced by cardiac vagal control. When compared to pre- or transition to menopause women, postmenopausal women have reduced cardiac function due to aging and hormone levels[1-3].

Regarding the diagnostic accuracy tests, the geometric index RRtri presented the potential to predict the last postmenopausal time. Besides, in our results, the greatest sensitivity values have been observed in the geometric indices, and this could be related to their ability to discriminate changes in autonomic modulation. Studies[6-9] showed that some linear and nonlinear HRV indices have good diagnostic accuracy in certain populations.

Table 3. Sensitivity, specificity, ROC curve, and positive and negative predictive values of heart rate variability indices to predict late postmenopausal.

Variables	ROC (95%CI)	p	Sensitivity	Specificity	PPV	NPV	Cutoff
MEANRR	0.57 (0.48–0.66)	0.157	48.53 (36.2–61.0)	70.91 (57.1–82.4)	1.67	0.73	>936.599975586
SDNN	0.59 (0.50–0.68)	0.0658	80.88 (69.5–89.4)	43.64 (30.3–57.7)	1.44	0.44	≤31.299999237
RMSSD	0.545 (0.453–0.635)	0.4008	60.29 (47.7–72.0)	56.36 (42.3–69.7)	1.38	0.70	≤21.200000763
RRtri	0.608 (0.516–0.695)	0.040[a]	88.24 (78.1–94.8)	32.73 (20.7–46.7)	1.31	0.36	≤9.666999817
TINN	0.583 (0.490–0.671)	0.1319	76.47 (64.6–85.9)	50.91 (37.1–64.6)	1.56	0.46	≤150
LF, ms²	0.563 (0.471–0.652)	0.2544	69.12 (56.7–79.8)	54.55 (40.6–68.0)	1.52	0.57	≤240
LF, nu	0.522 (0.430–0.613)	0.6817	52.94 (40.4–65.2)	63.64 (49.6–76.2)	1.46	0.74	≤58.299999237
HF, ms²	0.581 (0.489–0.669)	0.1319	75.00 (63.0–84.7)	47.27 (33.7–61.2)	1.42	0.53	≤233
HF, nu	0.520 (0.428–0.611)	0.7115	52.94 (40.4–65.2)	63.64 (49.6–76.2)	1.46	0.74	>41.099998474
LF/HF	0.521 (0.429–0.611)	0.6984	52.94 (40.4–65.2)	63.64 (49.6–76.2)	1.46	0.74	≤1.404000044
SD1	0.559 (0.467–0.649)	0.2718	58.82 (46.2–70.6)	58.18 (44.1–71.3)	1.41	0.71	≤14.800000191
SD2	0.600 (0.508–0.688)	0.0604	70.59 (58.3–81.0)	52.73 (38.8–66.3)	1.49	0.56	≤36.299999237
SD1/SD2	0.505 (0.413–0.596)	0.9310	17.65 (9.5–28.8)	67.27 (53.3–79.3)	0.54	1.22	>1.893000007
SAMPEN	0.544 (0.451–0.634)	0.4052	38.24 (26.7–50.8)	74.55 (61.0–85.3)	1.50	0.83	>1.827000022
ALFA1	0.545 (0.453–0.635)	0.3951	66.18 (53.7–77.2)	47.27 (33.7–61.2)	1.26	0.72	≤1.129999995
ALFA2	0.502 (0.411–0.594)	0.9645	67.65 (55.2–78.5)	45.45 (32.0–59.4)	1.24	0.71	≤0.587000012
ALFA1/ALFA2	0.508 (0.416–0.599)	0.8787	80.88 (69.5–89.4)	30.91 (19.1–44.8)	1.17	0.62	≤2.587248325

[a]p<0.05. 95%CI: 95% confidence interval; PPV: positive predictive value; NPV: negative predictive value; MEANRR: median of RR intervals/ms; SDNN: mean standard deviation of all normal RRis/ms; RMSSD: square root of the mean of squared differences between successive beat intervals/ms; HF: high frequency; LF: low frequency; LF/HF: low-frequency/high-frequency ratio; SD1: standard deviation of the RRis; SD2: standard deviation of long-term continuous RRis; SD1/SD2: ratio between short and long variations of the intervals; RRtri: triangular index; TINN: RRi triangular interpolation; SAMPEN: sample entropy; ALPHA1: short-term fractal scaling exponent; ALPHA2: long-term fractal scaling exponent; ALPHA1/ALPHA2: short-term fractal scaling exponent/long-term fractal scaling exponent ratio.

Out of various conventional HRV parameters, Hämmerle et al.[6] indicated the geometric index RRtri as an independent predictor of cardiovascular and all-cause mortality in a cohort of patients with atrial fibrillation. Silva et al.[7] observed that some geometrics indices showed better sensitivity and specificity for discriminating autonomic dysfunction in individuals with type 1 diabetes mellitus compared to individuals without the disease. Pivatelli et al.[8] reported that the parasympathetic indices presented the highest discriminatory power of coronary arterial disease, and among the nonlinear indices, only approximated entropy presented the highest discriminatory power. Using nonlinear dynamics methods, Corrêa et al.[9] found a significant difference between groups with and without pulmonary infections in the postoperative period of myocardial revascularization, through the cutoff levels set by the ROC curve for total DFA, entropy, and Lyapunov exponent, suggesting that these methods may have a prognostic value for these patients.

This study presents some limitations: (1) we only use HRV evaluation and did not perform other types of cardiovascular risk factors assessment, but this was the proposal to evaluate the predicted power of the HRV indices alone; (2) there was a difference in the age of the groups; however, due to the clinical characteristics of postmenopausal women, this was expected and had already been described in previous studies. Therefore, we do not believe that they could influence our findings; and (3) finally, there are limitations inherent to the study design, which does not allow establishing a cause-effect relationship of the outcomes.

Further studies are needed to monitor these postmenopausal phases for longer. The study of HRV, by being a noninvasive, low-cost, and risk-free method, can be clinically relevant in their diagnostic evaluation. The cutoff points in the HRV indices could be related to other physiologic parameters, such as quality of life and vasomotor symptoms, as well as being used as measures for interventions in this population, such as exercises and hormone therapy.

CONCLUSION

The geometric index RRtri was significantly lower in late postmenopausal women and presented the potential to predict late postmenopausal. The increase in postmenopausal time decreases global HRV indices.

AUTHORS' CONTRIBUTIONS

TDC: Data curation, Supervision, Writing – original draft. **ARN:** Data curation. **LSP:** Formal Analysis. **FRO:** Formal Analysis. **ECB:** Supervision. **JMSJ:** Conceptualization. **LCMV:** Conceptualization. **ICES:** Conceptualization, Supervision, Writing – review & editing.

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ORIGINAL ARTICLE

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Serum vascular endothelial growth factor as a marker for tubal pregnancy

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SUMMARY

OBJECTIVE: The objective of this study was to evaluate whether a single measurement of vascular endothelial growth factor could distinguish between intrauterine pregnancy and ectopic pregnancy and to correlate the levels of vascular endothelial growth factor with serum levels of progesterone and β-human chorionic gonadotropin in each subgroup.

METHODS: Ninety patients with a positive human chorionic gonadotropin test and either abdominal pain or vaginal bleeding were selected; pregnancies were singletons, spontaneously conceived, 42–56 days of gestational age. All patients had a transvaginal ultrasound examination and were divided into three subgroups: abnormal intrauterine pregnancy, tubal pregnancy, and normal intrauterine pregnancy. Tubal pregnancies were surgically treated and histologically confirmed. Blood samples were collected for the determination of β-human chorionic gonadotropin, progesterone, and vascular endothelial growth factor and their concentrations were compared in each subgroup. Receiver operating characteristic curve was calculated by comparing the subgroup of tubal pregnancy to the other groups. A Fisher discriminant function analysis was performed. The level of significance was 5%.

RESULTS: One-way analysis of variance revealed a significant correlation between the different subgroups and β-human chorionic gonadotropin, progesterone, and vascular endothelial growth factor serum levels ($p<0.001$). Vascular endothelial growth factor concentration was significantly higher for patients with tubal pregnancy than for other subgroups ($p<0.05$). β-Human chorionic gonadotropin and progesterone levels were higher in the subgroup with normal intrauterine pregnancies compared with the subgroups with tubal and abnormal intrauterine pregnancies ($p<0.05$). Serum vascular endothelial growth factor level >188.7 ng/mL predicted tubal pregnancy with 96.7% sensitivity, 95.0% specificity, 90.6% positive predictive value, and 98.3% negative predictive value.

CONCLUSIONS: Serum vascular endothelial growth factor could be a marker in discriminating intrauterine pregnancy from tubal pregnancy; its levels are increased in women with ectopic pregnancy compared with women with normal and abnormal intrauterine pregnancies.

KEYWORDS: VEGF. Pregnancy, ectopic. Vascular endothelial growth factor A. Trophoblasts. Pregnancy, tubal.

INTRODUCTION

Ectopic pregnancy (EP) is considered a true public health problem, as it is still a major cause of maternal morbidity and mortality, accounting for 9–13% of all pregnancy-related deaths[1]. Despite the introduction of highly sensitive assays for the estimation of serum human chorionic gonadotropin (hCG) and an increase in the sensitivity of transvaginal sonography (TVS), it is believed that 40–50% of cases initially are misdiagnosed[2].

Based on the combined use of TVS and serum hCG measurements, a variety of diagnostic algorithms have been proposed in the literature[3,4]; however, the utility of a single hCG measurement to confirm the absence of an EP has been questioned and the measurement of serial hCG values has been proposed[5,6]. Unfortunately, serial hCG values are not practical, especially when the patient presents for an emergency evaluation.

Vascular endothelial growth factor (VEGF) is a well-known angiogenic factor, which might play a key role in the establishment of a viable pregnancy, participating in the processes of implantation and placentation. That substance serves as a major modulator of vascular growth, remodeling,

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and permeability in endometrium, decidua, trophoblast, and also in the vascular development of the embryo[7,8]. The secretion and expression of VEGF is dependent on local conditions, such as hypoxia, and it has been observed that the cellular VEGF production is increased in hypoxic conditions[7-9]. The implantation environment in the oviduct is very different from that of well-vascularized endometrium, and the production and secretion of VEGF may be affected in the EP[10,11].

It would be particularly valuable if there was a reliable serum marker that could differentiate intrauterine pregnancy (IUP) from extrauterine pregnancy in a single measurement. In an emergency setup, it would decrease the time to diagnosis, reduce the possibility of tubal rupture, and diminish the maternal morbidity and mortality. The aim of the present study was (i) to evaluate whether a single measurement of VEGF would allow us to distinguish between IUP (normal and abnormal) and EP and (ii) to correlate the levels of VEGF with serum levels of progesterone and β-hCG in each subgroup.

METHODS

The study was approved by the Clinical Research Ethics Committee of the University of São Paulo.

Ninety patients were selected from a population of women presenting to the Hospital das Clínicas of the University of São Paulo Medical School from October 2006 to September 2007. Women elected had had a positive hCG test and presented with either abdominal pain or vaginal bleeding; all pregnancies were singletons, spontaneously conceived, with accurate assessments of their gestational age (42–56 days from the 1st day of the last menstrual period). A detailed informed consent was obtained from each patient before the inclusion.

All patients had a transvaginal ultrasound examination (Ecocee apparatus equipped with a 7.5 MHz transvaginal probe; Toshiba, Tokyo, Japan) and were divided into three subgroups: (i) abnormal (arrested) IUP (defined as a gestational sac greater than 16 mm of mean diameter without fetal tissue or an embryo greater than 5 mm without embryo cardiac activity); (ii) tubal pregnancy (no evidence of IUP, presence of a adnexal mass, and suboptimal rise of serum hCG levels in 48 h); all tubal pregnancies were surgically treated and were histologically confirmed; they did not receive any treatment with methotrexate before operation; (iii) normal IUP (intrauterine gestational sac, embryo vitality confirmed). Exclusion criterion was non-ampullar tubal pregnancy (surgically confirmed).

Blood samples were collected by peripheral venous puncture before treatment; a total of 15 mL blood was withdrawn (2 mL for β-hCG, 3 mL for progesterone, and 10 mL for VEGF determination). Blood samples for VEGF were collected in siliconized tubes and were allowed to coagulate at room temperature (RT) for 2–6 h; serum was obtained by centrifugation and stored at -80°C until assays were performed in batches. Serum VEGF was measured in triplicate by commercial ELISA (R&D System, Inc., Minneapolis, USA) specific for the human molecule. Samples were diluted in a ratio of 1:4 with assay diluent and incubated in triplicates in microtiter plates pre-coated with a monoclonal antibody specific for VEGF at RT for 2 h. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific for VEGF was added. After incubation at RT for 2 h and washing, a substrate solution was added. Color development was stopped after 20 min at RT and color intensity was read at 450 nm (reference wavelength 540 nm) within 30 min. Results were calculated from a standard curve (recombinant human VEGF165; range 15–1000 pg/mL) generated by a four-parameter logistic curve-fit and expressed as pg/mg cytosol protein. The sensitivity of the assay was <5.0 pg/mL; intra-assay variability was 5.1% at a VEGF concentration of 512 pg/mL.

Serum β-hCG was quantified with a two-site immunofluorimetric assay based on the direct sandwich technique (1235 AutoDELFIA Immunoassay System, AutoDELFIA hCG; PerkinElmer, Turku, Finland). The inter-assay and intra-assay coefficients of variation were 5.1 and 3.9, respectively. Serum progesterone was measured by a solid-phase RIA (1235 AutoDELFIA immunoassay system, AutoDELFIA progesterone; PerkinElmer Life and Analytical Sciences, Finland). The inter-assay and intra-assay coefficients of variation were 1.7 and 2.0, respectively. The sensitivity of the assay was 0.8 nmol/L.

The statistical analysis was performed using SPSS-PC software (version 13.0; SPSS, Chicago, Illinois, USA). Demographic data were compared using one-way analysis of variance (ANOVA) and serum concentrations of VEGF, β-hCG, and progesterone were compared in each subgroup using the Kruskal-Wallis test. Multiple comparisons were performed by nonparametric tests. A stepwise logistic regression model was used to select predictors of the tubal pregnancy subgroup. Receiver operating characteristic (ROC) curve was calculated to discriminate the tubal pregnancy subgroup from other groups. A Fisher discriminant function analysis was performed in order to classify the cases into the different subgroups. The level of significance was set at 5% for all tests.

RESULTS

The age of the patients ranged from 17 to 44 years [mean 29.6 ± (SD) 6.6 years]. A total of 41 (45.6%) patients were white and 49 (54.4%) were non-white. With respect to obstetric history, 18 (20.0%) patients were nulliparous and 8 (8.9%) had a history of EP in the contralateral Fallopian tube. There was no difference in maternal age between the three subgroups (p=0.633), but gestational age was significantly different between the subgroups (p=0.003).

Serum VEGF concentrations ranged from 15.6 to 783.1 ng/mL between all the subgroups. One-way ANOVA revealed a significant correlation between the different subgroups and β-hCG, progesterone, and VEGF serum levels (p<0.001) (Table 1). Serum VEGF concentrations were significantly higher for patients with tubal pregnancy compared with the other subgroups (p<0.05). β-hCG and progesterone levels were higher in the subgroup with normal intrauterine pregnancy compared with the subgroups with tubal and abnormal intrauterine pregnancies (p<0.05) (Table 2).

Multivariate logistic regression analysis was performed, and it showed that serum VEGF level, but neither β-hCG nor progesterone levels, could discriminate tubal pregnancies from intrauterine pregnancies.

Using the ROC curve, the threshold (VEGF concentration) was calculated for discriminating tubal pregnancy. The serum VEGF level that best predicted EP was 188.7 ng/mL, with this threshold value showing a sensitivity of 96.7%, a specificity of 95.0%, a positive predictive value (PPV) of 90.6%, and a negative predictive value of 98.3%. Cases with serum VEGF levels >188.7 ng/mL presented a greater chance of being classified as tubal pregnancy, with an odds ratio (OR)=551.0 [95% confidence interval (CI)=64.7–∞].

A Fisher discriminant function analysis was performed using VEGF, β-hCG, and progesterone levels, and the results are summarized in Table 3. The linear functions could predict the correct subgroup in 82.2% of cases.

DISCUSSION

The increase in the incidence of EP over the past years has been attributed to the growing number of risk factors such as a higher prevalence of sexually transmitted diseases, an increased tubal sterilization practice and subsequent attempted reversal, more frequent use of assisted reproduction technologies, late primiparity, and the use of levonorgestrel as an emergency contraceptive method[12-14].

Table 1. Serum VEGF, β-hCG, and progesterone concentrations.

	VEGF* (median ± SD)	β-hCG* median (range)	Progesterone* (median ± SD)
Tubal pregnancy	368.8 ± 167.7	4641 (108–46165)	6.1 ± 3.9
Evolutive intrauterine	83.6 ± 62.8	45944 (10124–239025)	22.5 ± 6.1
Non evolutive intrauterine	83.4 ± 51.3	6751 (190–76712)	9.7 ± 6.2

Kruskal-Wallis test. *p<0.001.

Table 2. Multiple comparisons between serum concentrations and different subgroups.

	Comparison	Z-value	p
VEGF	Tubal vs. Non-evolutive	6.5	**<0.001**
	Tubal vs. Evolutive	6.6	**<0.001**
	Non-evolutive vs. Evolutive	0.1	0.912
β-hCG	Tubal vs. Non-evolutive	-0.7	0.503
	Tubal vs. Evolutive	-6.0	**<0.001**
	Non-evolutive vs. Evolutive	-5.4	**<0.001**
Progesterone	Tubal vs. Non-evolutive	-1.8	0.070
	Tubal vs. Evolutive	-7.0	**<0.001**
	Non-evolutive vs. Evolutive	-5.2	**<0.001**

Nonparametric tests.
Bold indicates significance is p<0.05.

Table 3. Classification of function coefficients; vascular endothelial growth factor, β-hCG, and progesterone concentrations.

Constant	VEGF	β-hCG	Progesterone
Tubal - 8.1170	0.0335	-1.6×10^{-6}	0.2787
Evolutive - 10.8679	0.0113	-3.1×10^{-5}	0.7414
Non-evolutive - 3.1364	0.0092	-1.9×10^{-6}	0.3402

Fisher discriminant function analysis.

Pregnant patients presenting with vaginal bleeding as an emergency still represent a diagnostic challenge. Transvaginal ultrasound and serum β-hCG and serum progesterone determinations are the most widely used methods for EP diagnosis; nevertheless, ultrasound examination can be helpful just when an intrauterine gestation or an adnexal mass is seen and serial determinations of serum β-hCG can separate normal IUP from an abnormal IUP, but it cannot distinguish an abnormal IUP from an EP[15,16]. Progesterone concentrations are higher in women with normal IUP, but its application to differentiate an EP from an abnormal IUP is not reliable[17].

Vascular endothelial growth factor is indispensable for trophoblast development during vascular development of the[18]. In contrast to hCG and progesterone, which are trophoblast-dependent, this angiogenic factor is produced by both trophoblast and endometrium[9]. This difference is of extreme importance because the main discrimination between abnormal IUP and EP is not the viability of the trophoblast (reflected by low levels of both progesterone and hCG), but fundamentally in the ground of implantation.

Extrauterine implantation environments are very different from those of the endometrium and the hypoxic conditions at the unusual implantation site may cause increased VEGF production[19,20]. Lam P.M. measured the mRNA expression of VEGF and its receptor (KDR and ftl-1) in the implantation and non-implantation sites of the human Fallopian tubes with EP and described that the expression of VEGF was significantly higher in the implantation site of the tube with EP[21]. The authors suggested that VEGF may be the angiogenic factor responsible for the implantation of an EP in the oviduct.

We studied the value of a single measurement of VEGF for differentiating between ectopic and normal/abnormal IUP in a group of pregnant women with vaginal bleeding in the first trimester. As the different anatomic segments of the Fallopian tube are histologically distinct, we believe that different implantation conditions might influence the VEGF production by the trophoblast in each tubal portion; so, in the tubal pregnancy subgroup, only ampullary pregnancies were included, as they represent the main extrauterine site of trophoblast implantation[22,23]. Besides, we decided to delimit the gestational age (42–56 days), as the previous studies included larger periods (5–10 weeks), which might have biased the results. Serum VEGF concentrations ranged from 15.6 to 783.1 ng/mL between all the subgroups. Statistical analysis revealed that the concentration of VEGF was significantly different between the subgroups and that significantly higher values were observed in the patients with EP compared with IUP (normal and abnormal) subgroups ($p<0.001$). We found that serum VEGF levels >188.7 ng/mL could differentiate EP from intrauterine pregnancies with high sensitivity (96.7%), specificity (95.0%), PPV (90.6%), and NPV (98.3%). These data are in accordance with the results already published in the literature.

A prospective study performed in 20 patients with EP found that serum VEGF values were higher in women with EP when compared with those with abnormal IUP[10]. They postulated that serum VEGF levels were more specific and had a higher PPV than serum progesterone levels in differentiating the various types of pregnancies; therefore, this study supported the fact that a serum VEGF level of >200 ng/mL could distinguish intrauterine pregnancies from extrauterine pregnancies with a specificity of 90% and a PPV of 86% and abnormal intrauterine pregnancies from extrauterine pregnancies with a specificity of 80% and a PPV of 86%. These findings were confirmed by other authors; Felemban A. studied 45 pregnant women (EP, normal and abnormal intrauterine pregnancies – 15 cases each group) and stated that the cutoff concentrations of 200 pg/mL for VEGF could distinguish normal intrauterine pregnancies from EP with a sensitivity of 88%, a specificity of 100%, and a PPV of 100%[11]. Between EP and abnormal intrauterine pregnancies, the sensitivity was 87.5%, specificity was 75%, and PPV was 77.8%. A similar study described higher serum VEGF levels in women with EP than in women with intrauterine pregnancies of comparable gestational age[24].

As already shown in other studies, serum progesterone levels could differentiate abnormal (topic or ectopic) from evolutive intrauterine pregnancies. On the one hand, in the subgroup with evolutive intrauterine pregnancies, only 6.7% of cases had serum progesterone levels lower than 15 nmol/L.

On the other hand, progesterone levels were higher than 15.0 nmol/L in 13.3% of cases of the other two subgroups.

This study shows that serum VEGF, but not progesterone, could be a more specific marker in discriminating IUP from EP, as its levels are increased in women with EP compared with women with normal and abnormal intrauterine pregnancies.

This finding can allow earlier and more successful EP diagnosis and treatments in the emergency rooms. Indeed, VEGF antagonists are under investigation for their potential use in disorders characterized by pathological angiogenesis (such as tumor growth) and the inhibition of VEGF action may also be a potential medical treatment for EP[25]. This area deserves further investigation.

AUTHORS' CONTRIBUTIONS

FRC: Conceptualization, Data curation, Investigation, Methodology, Project administration, Writing – original & draft. **PPP:** Conceptualization, Writing – review & editing, Project administration, Validation, Visualization. **MAO:** Data curation, Writing – original & draft, Investigation. **RPVF:** Supervision, Validation, Visualization, Writing – review & editing.

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REVIEW ARTICLE

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Detection of atrial fibrosis using echocardiographic strain: a new pathway

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INTRODUCTION

Atrial fibrillation (AF) is the most common sustained cardiac arrhythmia, with a prevalence of approximately 3% in adults, which is likely to increase with age, making its prevention and appropriate management essential factors[1]. AF is independently associated with a 1.5- to 2-fold increase in morbidity and mortality risk by all causes in the population in general[2,3].

Inflammation has been associated with the pathophysiology of AF and several pathological processes, such as oxidative stress and apoptosis. Inflammation in the atrium seems to be related to the emergence of AF, which is part of the fibrosis pathophysiology, and contributes to its appearance in the left atrium (LA)[4].

Diseases such as ischemic cardiomyopathy, cardiac valvulopathy, and cardiac insufficiency are associated with the dilation of the LA and an increased risk of developing AF[1-3,5]. The enlargement of the LA was initially studied with the aim of evaluating its relationship with AF, because an atrium with a larger volume is associated with a higher risk of AF, mainly in the elderly[6]. Recently, a significant association of peak atrial longitudinal strain (PALS) of the LA and the progression of AF was demonstrated[4].

In addition to other cardiovascular diseases, the high prevalence of AF represents an important epidemiological, clinical, and economic concern. The identification of echocardiographic parameters, with the objective of an early detection of atrial alterations in structure and function, becomes a valuable tool that can contribute to the identification of patients with a higher risk or a worse prognosis in face of AF[6]. The increase in the LA fibrosis can predict the prognosis after ablation[7].

Echocardiography is the most used tool to evaluate atrial size and function[7]. As a diagnostic method, speckle tracking echocardiography (STE) has been used for the detection of atrial fibrosis and it presents good perspectives for its use in the routine of clinical practice[8]. STE is an advanced imaging technique that allows the assessment of the deformations of the LA reservoir function, potentially caused by the decrease in complacency due to atrial fibrosis[7].

The objective of this review was to investigate the usefulness of STE as an atrial fibrosis marker in patients with AF.

METHODS

This study is an integrative literature review carried out in six steps:

1) identification of the subject and selection of the research question;
2) definition of inclusion and exclusion criteria;
3) search for the studies and extraction of results;
4) assessment of the studies;
5) interpretation of the results; and
6) knowledge summarizing[9].

The research question was elaborated based on PICO search strategy (P – population: patients with FA; I – interest: atrial fibrosis; Co – context: STE for the assessment of atrial fibrosis). It resulted in the following guiding question: Are

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the parameters assessed in the STE useful as markers for atrial fibrosis in patients with AF?

The inclusion criteria were as follows: articles which the objectives aimed at discussing the use of STE as a marker for atrial fibrosis in patients with AF, of the type clinical studies, observational studies, and meta-analysis or systematic reviews that were published between 2011 and 2021. We excluded articles targeting the pediatric population, other review methods, studies that did not approach atrial fibrosis assessed by STE, books, monographs, dissertations, thesis, and editorials. Databases used were as follows: PubMed, ScienceDirect, *Literatura Latino Americana y del Caribe em Ciências de La Salud* (LILACS), *Medical Literature Analysis and Retrieval System Online* (MEDLINE), and *Portal da Biblioteca Virtual de Saúde* (BVS).

Sampling was carried out by survey and analysis of the publications using the descriptors selected from *Descritores em Ciências da Saúde* (DeCS, http://desc.bvs.br): Atrial fibrillation, Atrial fibrosis, Strain, Speckle tracking, and their respective translations to Portuguese language, with the crossing performed by the Boolean operator "*and*."

Initially, titles and abstracts were read by two independent researchers and each researcher registered the decision to include or not the study assessed (step 1). Divergent cases were submitted to a third researcher for evaluation (step 2). Subsequently, articles included by the three researchers were fully read in order to search for the study question (step 3).

The assessment with regard to the Level of Evidence (LE) followed the guidelines of the *Oxford Centre for Evidence-Based Medicine*[10,11] as shown in Table 1. Information extracted was descriptive and directly related to the study question (Tables 1 and 2).

The search retrieved 76 studies, which were reviewed according to the eligibility criteria, and 6 articles[5,8,12-15] were selected, as shown in Figure 1.

RESULTS

In total, six articles fulfilled the eligibility criteria and answered the research question. Figure 1 describes the flow for the identification, selection, eligibility, and inclusion of studies.

The articles were analyzed regarding their quality and categorized by the degree of recommendation and LE[11]. Two studies[8,12] were classified as recommendation grade A (high), two studies[13,14] were grade B (moderate), and two studies were grade C (low)[5,15]. With regard to the type of study, five articles were primary research and only one was a review (Table 1).

DISCUSSION

Atrial fibrosis causes conduction disturbances and contributes to the atrial remodeling. A recent study suggests that cardiomyocytes can release inflammatory cells, for instance, cardiac fibroblasts that are responsible for the fibrous tissue formation. They are activated by cytokines, growth factors, and adipokines, among others, being related to inflammatory diseases that can be aggravated when associated with comorbidities. Therefore, atrial inflammation, even the subclinical type, can contribute to the appearance of fibrosis[16].

In AF, an important LA remodeling occurs in addition to collagen deposition in the interstice. It causes fibrosis and consequently alterations in the electric conduction that tend to increase progressively, favoring the conversion to permanent AF[7]. Atrial

Table 1. Summary of studies according to author, year, level of evidence, method, and objective.

Author and year/ level of evidence and recommendation	Method	Objective
Nielsen et al., 2020[8]/1A	Systematic review and meta-analysis	Investigate whether the peak atrial longitudinal strain assessed by STE can predict AF recurrence after treatment with radiofrequency ablation.
Laish-Farkash et al., 2020[12]/1A	Clinical trial	Investigate the relationship between LA remodeling using STE and high-density voltage mapping in AF patients
Moreno-Ruiz et al., 2019[13]/2B	Cohort prospective	Evaluate the predictive value of PALS for arrhythmia recurrence after electrical cardioversion in persistent and long-standing persistent nonvalvular atrial fibrillation using STE
Leung et al., 2018[14]/3B	Case–control retrospective	Investigate the relationship between LA reservoir strain and total atrial conduction time using STE
Pilichowska et al., 2018[5]/2C	Observational prospective	Evaluate the relationship between LA fibrosis derived from STE parameters as well as biomarkers of fibrosis in patients with AF
Watanabe et al., 2015[15]/2C	Observational retrospective	Clarify the relationship between LA mechanical function STE and LA electrical remodeling using an electroanatomic mapping system and to estimate AF substrate

STE: speckle tracking echocardiography; AF: atrial fibrillation; LA: left atrium; PALS: peak atrial longitudinal strain.

Table 2. Summary of studies according to the number of patients and main results.

Number of patients	Main results
1,025[8]	– Patients with lower values of peak atrial longitudinal strain (PALS) are associated with an increased risk of AF recurrence. – PALS is considered a significant predictor of AF recurrence after radiofrequency ablation – PALS provides information regarding the expected response to ablation, which can be of use to select patients for optimal treatment.
42[12]	– Low-voltage zones ≥5% present a negative association with the LA reservoir phase, which suggests significant remodeling and fibrosis (p<0.01).
131[13]	– Atria from patients with AF of shorter evolution time are more compliant due to a lower degree of fibrosis when considering the difference in PALS values (p<0.001). – Patients with AF recurrence presented more fibrosis (remodeling) by lower global atrial longitudinal deformation (p<0.001).
602[14]	– Fibrosis is considered a primary driver of AF, resulting in the formation of micro reentry circuits that may initiate and perpetuate the atrial arrhythmia. – Remodeling of the atrial substrate and increasing fibrosis may reduce LA compliance (p<0.001). – LA compliance is impaired in AF, and together with the significant negative relation with total atrial activation time, suggests that these changes may be due to atrial fibrosis (p<0.001).
66[5]	– Left atrial diastolic (p<0.001) and volume (p<0.002) parameters correlate well with the extent of LA fibrosis, assessed by invasive methods. – STE may be useful in the noninvasive assessment of LA fibrosis (p<0.001).
52[15]	– Dyssynchrony was prominent in AF patients with low-voltage zones in the LA (p<0.001).

AF: atrial fibrillation; LA: left atrium; PALS: peak atrial longitudinal strain.

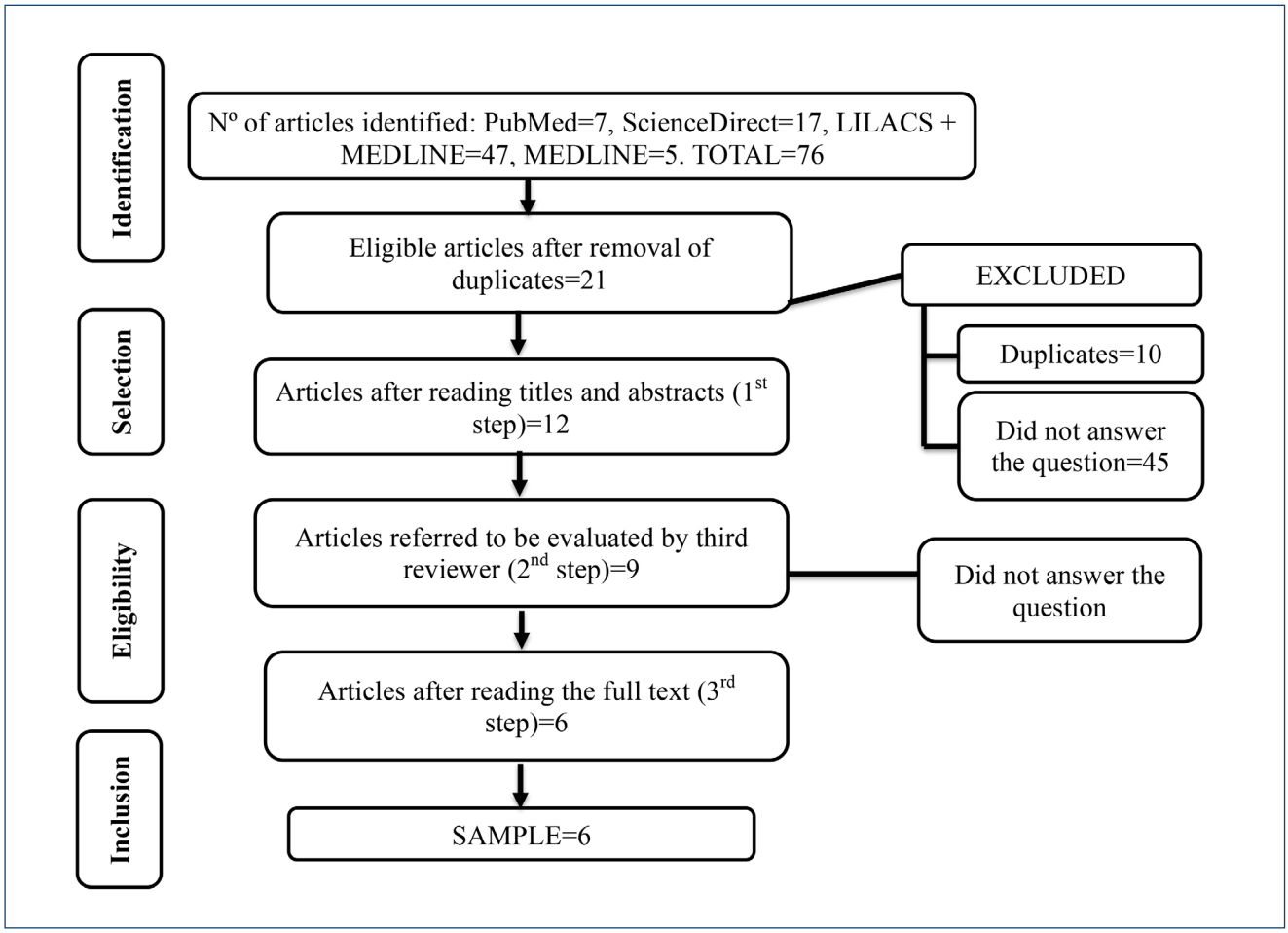

Figure 1. Flow diagram of search strategy results and study selection.

fibrosis is a result of an atrial structural remodeling and acts as a substrate for AF, playing an important role in the disease[17].

In the long run, fibrosis is an important factor causing mechanic damages[17]. It can be caused and/or aggravated by several diseases or clinical conditions, among them, AF itself, in which a rapid atrial myocyte depolarization occurs and contributes to the fibrosis[18]. Thus, AF may be a consequence of the fibrosis and can be aggravated by arrhythmia, leading to a chronic process.

Atrial fibroblast remodeling prevention is essential, and evidence shows how important it is to detect the fibrosis stage because it can help in the therapeutic decision for these patients[6]. Therefore, it has been suggested that the assessment of atrial fibrosis presence as early as possible is crucial.

Markers can be used in the clinical practice for the prediction, diagnosis, and prognosis, in addition to allowing the monitoring of the response to the treatments offered. It is noteworthy that markers should be used together with a critical analysis and always interpreted in the light of clinical data[19].

In the context of fibrosis and AF, the possibility of imaging examinations to identify and/or predict atrial fibrosis has emerged. STE is used to assess/track "stains" that are suggestive of myocardial deformation. In AF, the peak atrial longitudinal strain (PALS) measured at the end of reservoir phase is an important deformation parameter, since it depends essentially in the atrial compliance[6].

Nielsen et al.[8] suggest that PALS can be considered a superior predictor of AF recurrence after ablation because it reflects the compliance of the LA wall as well as atrial fibrosis and characterization and quantification of myocardial deformation (weighted mean difference [WMD]: 6.57, 95%CI −8.49 to −4.65, p<0.001).

The authors also defined an ideal PALS value to predict AF recurrence (<12.8%, range 10–18.8%), with a weighted mean sensitivity of 80% (range 74–86%) and specificity 87% (range 71–98%). The optimal value for PALS to predict the maintenance of sinus rhythm is >20.5% (range 15–30%), with a weighted mean sensitivity of 76% (range 56–97%) and specificity of 81% (range 58–100%)[8].

In a clinical trial carried out by Laish-Farkash et al.[12], the relationship between LA remodeling assessed by STE and high-density voltage mapping in patients with AF was investigated. The study showed low-voltage zones ≥5% were negatively correlated with LA reservoir phase, suggesting significant remodeling and fibrosis (p<0.01).

Evaluation of the fibrosis extension in the LA guided by magnetic resonance imaging may influence the decision-making process in the management of patients with AF, mainly by guiding the selection of patients considered adequate candidates for ablation and predicting the probability post-ablation of maintaining sinus rhythm[12]. This examination is an established tool for obtaining images of myocardial fibrosis; however, it is highly costly, requires experience for the appropriate image acquisition and analysis[7], cannot be performed in all patients (for instance, patients with chronic kidney insufficiency), and is not available in most hospitals in developing countries[6].

Moreno-Luiz et al.[13] carried out a study with the objective of assessing PALS predictive value in patients with persistent and long-standing persistent AF submitted to electrical cardioversion. The authors demonstrated that atria from patients with shorter evolution time AF are more compliant due to a lower degree of fibrosis when considering PALS values (p<0.001) and that patients with AF recurrence presented more fibrosis by lower global atrial longitudinal deformation (p<0.001).

Leung et al.[14] found out that, compared with controls, patients with paroxysmal AF and patients with persistent AF presented a progressive reduction in the LA reservoir deformation (36.9±11.6%, 29.8±13.4%, 24.2±12.3%, respectively, p<0.001). The study also demonstrated that both the presence and burden of AF were associated with morphofunctional abnormalities of the LA, represented by larger LA volumes, longer total atrial activation time, and more impaired LA reservoir strain.

Pilichowska et al.[7] reported that LA diastolic parameters derived from STE correlate well with the extent of LA fibrosis. Hence, they suggested STE could be useful in the noninvasive assessment of LA fibrosis and selection of candidates for ablation. In diagnosis and determining interventional AF treatment, the need for precise LA evaluation is highlighted because the LA wall properties are associated with the effectiveness of the treatment.

Watanabe et al.[15] suggested that LA dyssynchrony was especially pronounced in patients with paroxysmal AF who had a low-voltage zone in their LA (p<0.001). This alteration may be a result of the regional fibrosis of the LA myocardial tissue. Regional fibrosis may lead to the heterogeneity of LA wall, result in dyssynchrony, and also cause the local conduction delay by separating atrial myocytes.

Anamnesis, physical examination, and imaging examinations already established in the literature, associated with new examinations of atrial fibrosis markers, can represent the future of patients' assessment with or in risk of developing AF. The reason is that this strategy presents a potential to guide a more individualized and appropriate therapeutic choice for this disease, which represents a public health concern all over the world. In that regard, speckle tracking assessed by the echocardiography is an atrial fibrosis marker candidate useful for the selection of patients suitable for ablation.

CONCLUSION

Atrial fibrosis is considered a substrate for AF, especially in patients who are in an advanced stage of the disease. The use of markers is an important tool in the search for new means of disease management. In our review, we confirmed that STE can be considered a predictive, diagnostic, and prognostic marker for atrial fibrosis in patients with AF.

AUTHORS' CONTRIBUTIONS

MMBMS: Conceptualization, Data curation, Formal analysis, Writing – original draft. **DCO:** Conceptualization, Data curation, Formal analysis, Writing – original draft. **JVBC:** Data curation. **ATX:** Data curation. **LRC:** Data curation. **DJFN:** Data curation. **JMDC:** Writing – original draft, Writing – review & editing. **LRSV:** Writing – original draft, Writing – review & editing. **DCSF:** Writing – original draft, Writing – review & editing.

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REVIEW ARTICLE

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A narrative review on nonalcoholic fatty liver disease and nonalcoholic steatohepatitis versus hepatocellular carcinoma: do you mind?

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INTRODUCTION

Nonalcoholic fatty liver disease (NAFLD) is a predominant etiologic factor for liver disease. The risk of the development of hepatocellular carcinoma (HCC) in cases suffering from NAFLD has been stated[1]. Nonalcoholic steatohepatitis (NASH), a subtype of NAFLD, is a well-known etiologic factor for cirrhosis and it is one of the risk factors for the development of HCC[2]. In addition, the association of obesity with NAFLD is well established and augmentation in obesity has been associated with an increase in the incidence of HCC[1,3].

In the present review, the interrelation of NAFLD and HCC, obesity and NAFLD, and obesity and HCC has been purposed to be discussed by taking into consideration the relevant prevalence, clinical evidence, pathogenic mechanisms, and diagnostic parameters.

METHODS

We have performed searching the data from online databases such as PubMed/MEDLINE and Google Scholar by using the following key words: nonalcoholic fatty liver disease/NAFLD, hepatocellular carcinoma/HCC, obesity, and NASH. The data have been included in various studies and review articles.

An association of non-alcoholic fatty liver disease and hepatocellular carcinoma

Prevalence

A review of worldwide data indicates that NAFLD is a prevalent chronic disorder and different types of data reveal its prevalence ranging from 6% to 35%[4]. Some of the cases with NAFLD land up in the complication "HCC," as the data from a meta-analysis by Younossi et al.[1] indicate that the incidence of HCC is 0.44 per 1,000 person-years. However, liver cirrhosis is a complication of NASH and it further leads to HCC. Ascha et al.[2] reported that HCC was found in 2.6% of cases suffering from NASH-associated cirrhosis[1]. In addition, the increased death rate has been reported in cases suffering from NASH due to HCC, especially in NASH patients suffering from advanced fibrosis. Hashimoto et al.[5] reported a survival rate of nearly 83% in 5 years in those suffering from HCC, especially in NASH cases suffering from advanced fibrosis[5]. The steatohepatitic hepatocellular carcinoma (SH-HCC) variant was noted in approximately 35% cases of HCC: a distinctive histological variant of HCC in hepatitis C virus related cirrhosis associated with NAFLD/NASH[6].

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Clinical evidence

As per a retrospective cohort study conducted in a large number of patients by Kanwal et al.[7], the prevalence of HCC was higher in patients, particularly in those who were suffering from NAFLD, and the incidence rate of HCC was significantly augmented in NAFLD patients with cirrhosis. In that study, nearly 297,000 NAFLD cases were compared with a similar number of controls. Nearly 500 patients with NAFLD developed HCC (0.21/1,000 person-years). In addition, an association of NAFLD or NASH and an increased risk of HCC was established in the systematic review by White et al.[8], and the risk of HCC was particularly higher in cirrhotic NAFLD or NASH cases. Researchers reviewed a total of 57 studies (cohort studies, case-control, cross-sectional studies, and case series) to establish this association[8]. The results of a meta-analysis of a large cohort study, including approximately 19 million people with approximately 137,000 NAFLD/NASH patients, revealed higher risks of cirrhosis and HCC in the NAFLD/NASH cases and significantly increased risks of cirrhosis and HCC in the NASH cases with high-risk Fib-4 scores[9]. In addition, Bugianesi et al.[10] reported a predominantly increased numerical value of the NASH in HCC cases with cryptogenic cirrhosis, by comparing the HCC cases with viral and alcohol-associated liver disorders.

Pathogenesis

The pathogenesis of HCC from NAFLD was elaborated by Takakura et al.[11], including the following steps and/or aspects:

i. The progression of NASH to HCC was associated with an inflammatory pathway inclusive of tumor necrosis factor (TNF).
ii. A diet containing a higher amount of fat leads to increased stress in the endoplasmic reticulum (ER).
iii. Increased stress in the ER leads to increased lipogenesis or steatosis in the liver.
iv. Increased stress in the ER and lipogenesis contributes to the increased synthesis of reactive oxygen species (ROS) that result in the rise in oxidative stress and contributes to genomic instability.
v. Increased ER stress coupled with oxidative stress activates inflammatory mediators.
vi. Macrophages release TNF that causes hepatocyte proliferation and expansion of HCC progenitors.
vii. TNF further activates chemokines and growth factors/cytokines.
viii. Dysbiosis leads to liver fibrosis and carcinogenesis through the involvement of the microbiota-liver axis.
ix. Intestinal microbiota and pathogen-associated molecular patterns (PAMPs) are etiological factors for liver disorders through the route of the portal vein.
x. Patients with NAFLD and NASH have raised intestinal permeability and excessive growth of intestinal bacteria.
xi. The synthesis of TNF-α in Kupffer cells is stimulated by lipopolysaccharide (LPS) in portal blood through a rise in the Toll-like receptor 4 (TLR4) signal.
xii. Increased sensitivity to transforming growth factor-beta (TGF-β) by LPS leads to the development of liver fibrosis and tumorigenesis in the liver.
xiii. Increased levels of deoxycholic acid (DCA) contribute to the senescence of stellate cells which further causes hepatocarcinogenesis via the senescence-associated secretory phenotype (SASP) factor.
xiv. Pathways of STAT-1-dependent NASH and STAT-3-dependent HCC from fatty liver.
xv. Pro-tumorigenic signaling pathways are stimulated in HCC.
xvi. Signal transducer and activator of transcription-3 (STAT-3) signaling leads to the transformation of tumor progenitors and progression to HCC.
xvii. STAT-3 suppresses the activity of T cell protein tyrosine phosphatase (TCPTP) that leads to HCC in obesity.

In addition, older age and advanced fibrosis are predominant risk factors for HCC; therefore, ongoing screening for HCC is reported as being essential for NASH patients with advanced fibrosis[5].

An association of obesity and non-alcoholic fatty liver disease

Prevalence

As per a meta-analysis of 86 studies from 22 countries, the prevalence of obesity among patients with NAFLD was projected at 51% by Younossi et al.[1]. As per a review by Milić et al.[12], NAFLD is the most prevalent liver disease worldwide. NAFLD is demonstrated as either steatosis or NASH, and central abdominal obesity contributes to the causation processes of NAFLD; thus, it is reported that up to 80% of cases with NAFLD are obese[12].

Clinical evidence

Overweight and obesity, per se, are stated as a kind of legacy for the accrued prevalence of NAFLD in metabolically healthy

men and women as an outcome of a large cohort study conducted in nearly 77,000 men and women free of NAFLD and metabolic abnormalities at baseline. Nearly 10,000 participants developed NAFLD with a prevalence rate of approximately 30/1,000 person-years[13]. A study conducted by Bertola et al.[14] reported upregulation of genes associated with inflammatory and immune response and that further leads to T-cell stimulation in morbidly obese patients by serum palmitate stimulation of the TLR pathway that contributes to the development of steatosis.

Pathogenesis

The pathogenesis of obesity and NAFLD was elaborated by Jennifer et al.[15] as follows: Researchers have reported that obesity is a major or at least a contributing risk factor for NAFLD. The National Health and Nutrition Examination Survey III stated that nearly 7% of lean adults and approximately 28% of overweight/obese adults had hepatic steatosis, and the visceral adipose tissue (VAT) is predominantly associated with NAFLD. It is reported that VAT is significantly related to liver inflammation and fibrosis. The hormonal and biological activity of adipose tissue through secretion of adipokines by white adipocytes, the release of inflammatory cytokines by macrophages within adipose tissue, and the release of free fatty acids from adipocytes are increasingly recognized as contributing to insulin resistance (IR) and metabolic disease, including NASH. The following factors lead to IR, NAFLD, and NASH: i) the hormonal and biological actions of adipose tissue through secretion of adipokines by white adipocytes, ii) secretion of inflammatory cytokines by macrophages within adipose tissue, and iii) secretion of free fatty acids from adipocytes. As per a review of different data by Milić et al.[12], VAT leads to a rising incidence of NAFLD in morbidly obese cases. It has also been reported that secretion of adipokines from VAT as well as lipid accumulation in the liver further promote inflammation through nuclear factor kappa B signaling pathways that are stimulated by free fatty acids, leading to IR[12].

In obese individuals, stored fat in the abdomen has an impact on the metabolism of fat and glucose and stored fat-containing liver is insulin-resistant. IR is often associated with chronic inflammation, and numerous mediators released from immune cells and adipocytes are propounded as the causative factors for IR. These mediators have also been expressed to contribute to the development of NAFLD[16]. We also currently emphasized possible metabolic, environmental, and genetic associations of NAFLD versus HCC[17]. Finally, NASH is also connected with cryptogenic cirrhosis particularly in the elderly patients with type 2 diabetes mellitus and obesity[18].

CONCLUSIONS

Of note, a significantly increased number of HCC patients with cryptogenic liver disease had well-differentiated tumors than in HCC patients with chronic viral hepatitis and alcoholism. As such, HCC is one of the debilitating complications of NAFLD/NASH and obesity is a causative factor for NAFLD/NASH. Thus, various preclinical and clinical data suggest that obesity appears to be an important causative factor in the progression of NAFLD/NASH to HCC.

AUTHORS' CONTRIBUTIONS

DT: Conceptualization, Data curation, Formal Analysis, Investigation, Methodology, Project administration, Resources, Software, Validation, Visualization, Writing – original draft. **IS:** Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – review & editing. **AP:** Conceptualization, Data curation, Formal Analysis, Investigation, Methodology, Project administration, Resources, Software, Validation, Visualization, Writing – original draft. **DS:** Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – review & editing. **PV:** Conceptualization, Data curation, Formal Analysis, Investigation, Methodology, Project administration, Resources, Software, Validation, Visualization, Writing – original draft. **PI:** Conceptualization, Data curation, Formal Analysis, Investigation, Methodology, Project administration, Resources, Software, Validation, Visualization, Writing – original draft. **JR:** Conceptualization, Data curation, Formal Analysis, Investigation, Methodology, Project administration, Resources, Software, Validation, Visualization, Writing – original draft. **CK:** Validation, Visualization, Writing – review & editing.

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COMMENTARY

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Comment on "Orthostatic changes in blood pressure and survival in elderly cardiopaths"

Ming Zhao[1]

Dear Editor,

We were very pleased to read the article entitled "Orthostatic changes in blood pressure and survival in elderly cardiopaths" by Wang and his colleagues[1]. In this study, the authors revealed that there was a low frequency of orthostatic hypotension (OH) and a mild high frequency of orthostatic hypertension when compared with previous studies, and no association was observed with overall mortality or with the survival time of elderly patients with heart disease. However, some concerns should be raised according to my opinion.

The main problem of the study was that taking central nervous system acting medications was not considered an exclusion criterion. Several psychoactive medications may alter the blood pressure response to standing, leading to drug-related orthostatic hypotension (OH) (Figure 1)[2]. A study found that OH develops in up to 40% of patients taking antipsychotics[3]. Thus, exclusion criteria need to include patients taking cardiovascular drugs and patients taking central nervous system drugs.

Therefore, both cardiovascular drugs and central nervous system drugs can increase the risk of OH. Medical therapy is one of the most common causes of OH. When considering OH, both groups should be excluded.

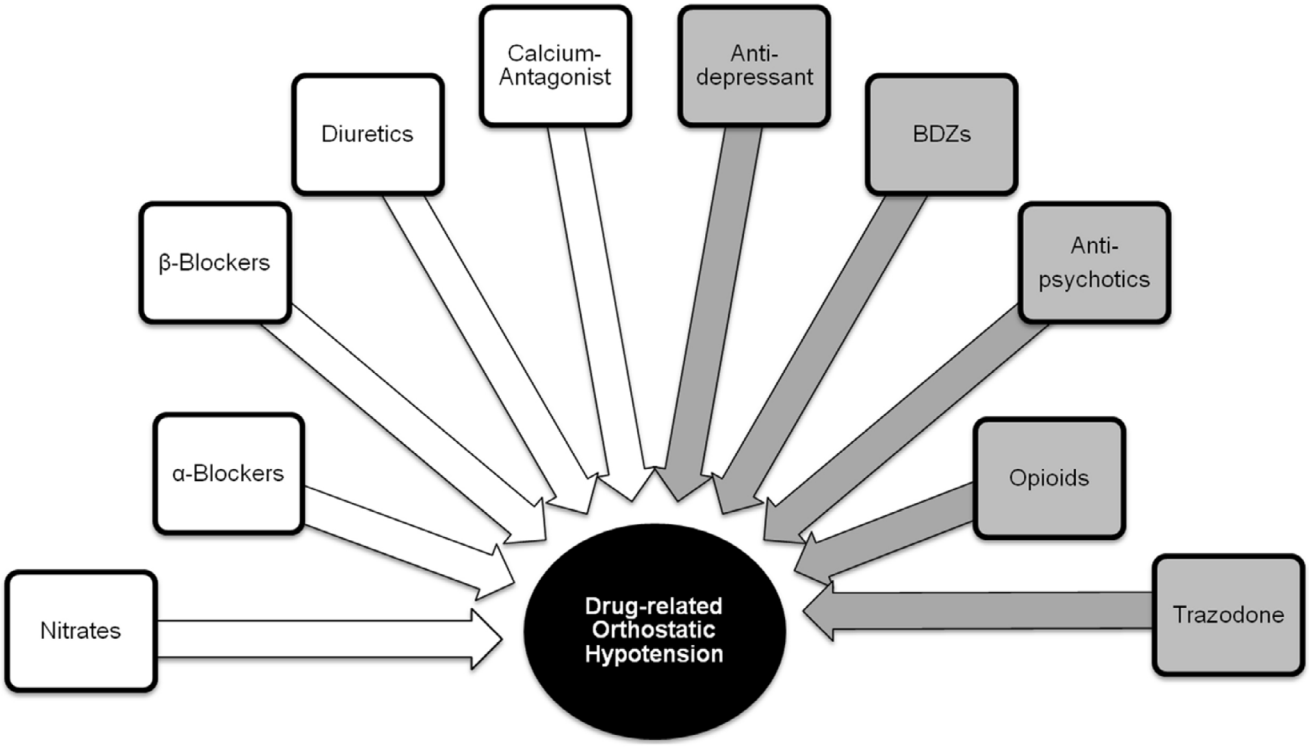

Figure 1. Medications acting on cardiovascular system (white) and central nervous system (dark gray) potentially responsible for drug-related orthostatic hypotension[2].

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