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Minimum minimorum: thyroid minimally invasive FNA, less is more concept? *Volens nolens?*

Demet Sengul[1*], Ilker Sengul[2,3]

A Deucalione, diagnosis for the crucial endocrine organ, the thyroid, remains significant to date. *Bonitas non est pessimis esse meliorem.* Furthermore, the butterfly-shaped gland, being necessitated a delicate deal, is apparent[1-3]. In thyroidology, image-guided interventional techniques have globally been noticed and increasingly harnessed over the past four decades[4]. *Bene diagnoscitur, bene curatur.* Having said that, a dynamic discipline, thyroid cytopathology, still harbors a highly controversial issue, *id est*, indeterminate cytology, resulting in an ongoing debate[4-11]. Despite the roles of sonography and fine-needle aspiration (FNA) are well-established globally for contributing to rule out blurred lines[5-8] in the diagnosis of thyroid nodules, to the best of our knowledge, the ideal needle size has not been stated in a released, well-accepted management guideline to date[1-3,12-16]. However, the adequacy of the finer and thicker needles has been proclaimed as similar by many authors[13], though the latter has unfortunately been utilized frequently. We have reported an FNA serial of *"non Deucalione, sed, a decennium"* by the surgeon-performed ultrasonography (SUS) with the 27-G needles with a reasonable rate of nondiagnostic cytology. Will it be evaluated and accepted as "less is more concept"? *Volens nolens?*

We had utilized the additional administration of preprocedural topical and local anesthesia in SUS-based serial during this decade and, *ad hoc*, have currently presented and recommended a novel term, "Thyroid minimally invasive FNA" (*Thy MIFNA*)[1–3,11], contributing in thyroidology. *Minimum minimorum? Adequatio intellectus et rei? Dum vivimus servimus.*

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AUTHORS' CONTRIBUTIONS

DS: conceptualization, data curation, formal analysis, investigation, methodology, project administration, resources, software, supervision, validation, visualization, writing – original draft, and writing – review and editing. **IS:** conceptualization, data curation, formal analysis, investigation, methodology, project administration, resources, software, validation, visualization, writing – original draft, and writing – review and editing.

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Endoscopic biliary sphincterotomy: electric current mode

Mateus Pereira Funari[1*], Vitor Massaro Takamatsu Sagae[1], Eduardo Guimarães Hourneaux de Moura[1], Wanderley Marques Bernardo[1]

The Guidelines Project, an initiative of the Brazilian Medical Association, aims to combine information from the medical field to standardize how to conduct and assist in the reasoning and decision-making of doctors. The information provided by this project must be critically evaluated by the physician responsible for the conduct that will be adopted, depending on the clinical condition of each patient.

Guideline conclusion: April 2021.

Societies: Sociedade Brasileira de Endoscopia Digestiva.

Group AMB: Wanderley Marques Bernardo

INTRODUCTION

Endoscopic retrograde cholangiopancreatography (ERCP) associated with biliary sphincterotomy is a procedure performed widely in medical practice. However, this intervention is not an exempt from complications (4–5%) such as acute pancreatitis, bleeding, perforation, cholangitis, or even death (0.02–0.4%)[1-3].

Several studies point to the correlation between the electric current mode (pure cut, blend, pulsed cut, or endocut, and pure cut followed by blend) used in endoscopic sphincterotomy and the incidence of adverse events[4-6]. A better knowledge of the subject based on evidence can assist us in making the best decision in clinical practice.

Our objective is, through a systematic review and meta-analysis, to trace the safety profile of each modality of electric current (pure cut, pulsed cut, blend cut, and pure cut followed by blend) employed in endoscopic biliary sphincterotomy to reduce the incidence of adverse events related to this procedure.

METHODS

A systematic review and meta-analysis of the literature (Medline, Central Cochrane, Embase, LILACS VHL, and grey literature) were carried out according to the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analysis) recommendations. We used the PICO system (Patient: older than 18 years with the indication of ERCP and biliary sphincterotomy; Intervention and Control: respective modalities of electric current; and Outcome: adverse events such as acute pancreatitis, bleeding, perforation, and cholangitis).

We selected only randomized controlled trials that included patients aged more than 18 years who underwent ERCP with biliary sphincterotomy for various causes (e.g., choledocholithiasis, obstructive neoplasia, benign strictures, and biliary fistulas) randomized to any of the modalities of electric current under evaluation.

The risk of bias in each study was assessed using the Cochrane bias risk tool[8]. The level of evidence for each outcome was evaluated according to GRADE (Grading of Recommendations Assessment, Development and Evaluation)[9].

The data were meta-analyzed using the RevMan 5.3 software, and the results were revealed as forest plots.

RESULTS

After removing duplicates, 12,282 articles were screened, including 10 randomized clinical trials in our study[4,10-18]. Annex Figure 1 summarizes the selection process.

The risk of bias in the included studies is expressed in Annex Table 1.

The characteristics of the studies and results are presented in Chart 1.

Results expressed by comparison:

ENDOCUT VS. *BLEND*

→ Inclusion of two studies with a total of 460 patients[4,12].

Acute pancreatitis

There was no difference between groups for pancreatitis in general (RD 0.01 [-0.03; 0.04], p = 0.62, I^2 = 48%) or in the mild, moderate, and severe subgroups.

Moderate level of certainty.

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Chart 1. Summary of study characteristics and results.

Study	N	Compared groups	ERCP indications	Age (MEAN)	Gender (M/F)	Homogeneous groups	outcomes
Tanaka, 2015	360	Endocut (200)	Choledocolithiasis and CBD stenosis (Benign and malignant)	73 (23-101)	56%/44%	Yes	**Pancreatitis:** 5 mild; 1 moderate; 0 severe **Bleeding:** 14 mild; 0 moderate; 0 severe
		Blend (160)					**Pancreatitis:** 7 mild; 0 moderate; 0 severe **Bleeding:** 18 mild; 0 moderate; 0 severe
Norton, 2005	267	Endocut (134)	Choledocolithiasis, stenosis (benign and malignant), SOD, PSC	59 (19-99)	47%/51%	Yes	**Pancreatitis:** 1 mild; 2 moderate; 0 severe **Bleeding:** 4 mild; 4 moderate; 0 severe
		Pure cut (133)					**Pancreatitis:** 1 mild; 0 moderate; 0 severe **Bleeding:** 29 mild; 6 moderate; 0 severe
Macintosh, 2004	246	Pure cut (116)	Choledocolithiasis, SOD, pancreatitis, stent insertion	56.4	31%/69%	Yes	**Pancreatitis:** 5 mild; 2 moderate; 2 severe **Bleeding:** 54 mild; 6 moderate; 1 severe
		Blend (130)					**Pancreatitis:** 5 mild; 3 moderate; 0 severe **Bleeding:** 26 mild; 6 moderate; 1 severe
Kida, 2004	84	Endocut (41)	Choledocolithiasis, malignant strictures, others	66.2	53%/47%	Yes	**Pancreatitis:** 4 (total) **Bleeding:** 12 mild; 1 moderate; 0 severe
		Pure cut (43)					**Pancreatitis:** 1 (total) **Bleeding:** 22 mild; 6 moderate; 0 severe
Stefanidis, 2003	186	Pure cut (62)	Choledocolithiasis	64 (40-86)	56%/44%	Yes	**Pancreatitis:** 2 mild; 0 moderate; 0 severe **Bleeding:** 18 mild; 2 moderate; 1 severe
		Blend (62)					**Pancreatitis:** 8 mild; 0 moderate; 0 severe **Bleeding:** 6 mild; 0 moderate; 1 severe
		Pure cut followed by blend (62)					**Pancreatitis:** 8 mild; 0 moderate; 0 severe **Bleeding:** 9 mild; 0 moderate; 1 severe
Wasim, 2001	86	Endocut (55)	Choledocolithiasis, SOD, Obstructive jaundice and pancreatitis	Unclear	Unclear	Unclear	**Pancreatitis:** 1 mild; 3 moderate; 1 severe **Bleeding:** 1 (total)
		Pure cut (31)					**Pancreatitis:** 0 mild; 0 moderate; 0 severe **Bleeding:** 0 (total)
Gorelick, 2001	142	Pure cut (75)	Choledocolithiasis, SOD, biliary stricture, bile leak	Unclear	30%/70%	Yes	**Pancreatitis:** 6 mild; 6 moderate; 2 severe **Bleeding:** 4 mild; 3 moderate; 1 severe
		Pure cut followed by blend (67)					**Pancreatitis:** 5 mild; 5 moderate; 1 severe **Bleeding:** 5 mild; 1 moderate; 0 severe

Continue...

Chart 1. Continuation.

Study	N	Compared groups	ERCP indications	Age (MEAN)	Gender (M/F)	Homogeneous groups	outcomes
Sanjiv, 2000	50	Pure cut (17)	Unclear (but the author states that the indication was homogeneous among groups)	70	Unclear	Yes	**Pancreatitis:** 0 mild; 0 moderate; 0 severe **Bleeding:** 0 moderate; 0 severe
		Blend (15)					**Pancreatitis:** 0 mild; 0 moderate; 0 severe **Bleeding:** 0 moderate; 1 severe
		Pure cut followed by blend (18)					**Pancreatitis:** 0 mild; 0 moderate; 1 severe **Bleeding:** 0 moderate; 0 severe
Kohler, 1998	100	Endocut (50)	Choledocolithiasis, chronic pancreatitis, CBD strictures, miscellaneous	Homogeneous	Homogeneous	Yes	**Pancreatitis:** 4 mild; 0 moderate; 0 severe **Bleeding:** 2 mild; 0 moderate; 0 severe
		Blend (50)					**Pancreatitis:** 1 mild; 0 moderate; 0 severe **Bleeding:** 13 mild; 0 moderate; 0 severe
ELTA, 1998	170	Pure cut (86)	Choledocolithiasis, SOD, stent insertion, miscellaneous	53 (19-96)	35%/65%	Yes	**Pancreatitis:** 3 mild; 0 moderate; 0 severe **Bleeding:** 0 mild; 0 moderate;1 severe
		Blend (85)					**Pancreatitis:** 7 mild; 2 moderate; 1 severe **Bleeding:** 0 mild; 0 moderate; 1 severe
Siegel, 1994	100	Bipolar (50)	Unclear	Unclear	Unclear	Unclear	**Pancreatitis:** 0 (total)
		Monopolar (50)					**Pancreatitis:** 6 (total)

n = Number of patients included; **compared groups** = in parentheses, we have the number of patients randomized to each group; **age** = mean expressed in years with the range in parentheses when provided in the study; **SOD** = Sphincter of oddi disfunction; **PSC** = Primary sclerosing cholangitis; **CBD** = common bile duct

Bleeding

There was no difference between groups for bleeding in general (RD -0.11 [-0.31; 0.08], p = 0.27, I^2 = 86%) or in the mild, moderate, and severe subgroups.

Very low level of certainty.

Perforation

No difference between groups (absence of cases in both arms).

Moderate level of certainty.

ENDOCUT VS. PURE CUT

→ Inclusion of three studies with a total of 437 patients[11,14,18].

Acute pancreatitis

There was no difference between groups for pancreatitis in general (RD 0.05 [-0.01; 0.11], p = 0.12, I^2 = 57%) or in the mild, moderate, and severe subgroups. In the total of pancreatitis episodes, three studies individually presented more events in the pulsed cut group; however, due to the high heterogeneity, the random effect was used, with no difference between the analyzed arms.

Low level of certainty.

Bleeding

More bleeding in general was observed in the pure cut group (RD -0.19 [-0.25; -0.12], p < 0.00001, I^2 = 96%). This difference

was due to self-limited (mild) bleeding (RD -0.23 [-0.31; -0.15], p < 0.00001, I^2 = 34%), with no difference in the incidence of moderate (RD -0.05 [-0.15; 0.05], p = 0.3, I^2 = 64%) or severe cases (RD 0.00 [-0.02; 0.02], p = 1, I^2 = 0%).

Moderate level of certainty.

Cholangitis

No difference among groups (RD -0.01 [-0.09; 0.06], p = 0.7).

Low level of certainty.

Perforation

Absence of difference between the groups (RD 0.00 [-0.01; 0.02], p = 0.7, I^2 = 0%).

Low level of certainty.

PURE CUT VS. *BLEND*

→ Inclusion of four studies with a total of 572 patients[10,13,15,16].

Acute pancreatitis

Absence of difference for pancreatitis in general (RD -0.03 [-0.07; 0.01], p = 0.17, I^2 = 32%) and in mild (RD -0.03 [-0.07; 0.00], p = 0.08, I^2 = 33%), moderate (RD -0.01 [-0.03; 0.01], p = 0.38, I^2 = 0%), and severe subgroups (RD -0.00 [-0.01; 0.02], p = 0.68, I^2 = 0%).

Low level of certainty.

Bleeding

More bleeding in general was observed in the pure cut group (RD 0.26 [0.61; 0.35], p < 0.00001, I^2 = 0%). This difference was based on self-limited (mild) bleeding (RD 0.24 [0.15; 0.33], p < 0.00001, I^2 = 0%), without difference in moderate (RD 0.01 [-0.02; 0.04], p = 0.51, I^2 = 0%) or severe cases (RD -0.00 [-0.02; 0.02], p = 0.73, I^2 = 0%).

High level of certainty.

Cholangitis

Absence of difference among groups (p = 0.47).

Low level of certainty.

PURE CUT FOLLOWED BY BLEND VS. *BLEND*

→ Inclusion of three studies with a total of 301 patients[15-17].

Acute pancreatitis

Absence of difference for pancreatitis in general (RD 0.06 [-0.02; 0.13], p = 0.12, I^2 = 0%) and in mild (RD 0.04 [-0.02; 0, 10], p = 0.15, I^2 = 23%), moderate (RD 0.00 [-0.04; 0.05], p = 0.91, I^2 = 0%), and severe subgroups (RD 0.01 [-0.02; 0.04], p = 0.45, I^2 = 0%).

Low level of certainty.

Bleeding

Absence of difference for bleeding in general (RD -0.10 [-0.24; 0.04], p = 0.18, I^2 = 61%) and in the mild, moderate, and severe subgroups.

Low level of certainty.

PURE CUT FOLLOWED BY BLEND VS. PURE CUT

→ Inclusion of two studies with a total of 157 patients[15,17].

Acute pancreatitis

Absence of difference for pancreatitis in general (RD -0.01 [-0.11; 0.09], p = 0.82, I^2 = 0%) and in the mild, moderate, and severe subgroups.

Low level of certainty.

Bleeding

Absence of difference in the incidence of mild (RD -0.05 [-0.16; 0.07], p = 0.41, I^2 = 0%), moderate (RD 0.00 [-0.04; 0,04], p = 1.0, I^2 = 0%), or severe bleeding (RD 0.01 [-0.04; 0.06], p = 0.58, I^2 = 0%).

Moderate level of certainty.

DISCUSSION

Mixed current modes (pulsed or endocut and blend) have greater coagulation power when compared to pure cut[4,19]. For this reason, they have been used to prevent bleeding during endoscopic biliary sphincterotomy. However, its greater coagulation power causes deeper dissemination of thermal energy to adjacent tissues and, in the case of biliary sphincterotomy, it is questioned whether this can increase the incidence of acute pancreatitis after ERCP.

While comparing endocut and blend with pure cut, we noted a similar profile, with more cases of pancreatitis in the arms of the mixed mode, but without statistical significance. It is possible that new studies, with an increased sample size, reveal a difference among the methods. It is worth remembering that one of the included studies was interrupted early due to the high incidence of pancreatitis in the arm that used the blend[10].

Regarding bleeding, mixed currents were found to be superior only in cases of self-limited bleeding (considered mild),

with no difference in the incidence of clinically significant bleeding (moderate and severe).

A strategy described to prevent pancreatitis and bleeding is to begin the incision with pure cut (due to its proximity to the pancreatic duct) and to proceed with a mixed current (due to its proximity to thicker vessels). We included three studies that used this strategy, using the blend mode at the end of the incision[15-17]. However, there was no difference in the incidence of acute pancreatitis or bleeding when compared to the pure cut or blend used throughout the incision.

Cholangitis and perforation are uncommon adverse events and have no apparent relation to the electric current modality used in biliary sphincterotomy.

This guideline has some limitations, such as heterogeneity in the bleeding definition, which was circumvented with a new definition applied to each study individually. Another point is the inclusion of articles available only as abstract; however, in these cases, all the outcomes of interest were available. In addition, there is a difference in the inclusion criteria of studies, though the interference of this factor is neutralized by the fact that we included only randomized studies with homogeneous groups. Another limitation is the use of relatively old electrosurgical units in the included studies, which are not used at present in many endoscopic units; however, this is also mitigated by the fact that the principle of each modality of electric current remains in different units, although these strategies are not so modern.

This guideline has a great evidence level since it includes only randomized clinical trials with homogeneous groups in each study. The highly sensitive and systematic literature review followed by the meta-analysis allows us to face the highest level of evidence possible with the current literature.

There is no ideal electric current mode for all situations or sufficient evidence in the literature to recommend one method over others. It is essential to know the effect of each modality to prevent adverse events. We concluded that there is no ideal electric current modality to prevent all adverse events; however, it is essential to understand their respective mechanisms of action and the risk factors of each patient for the endoscopist to make the best decision in clinical practice.

Factors such as the lack of access to information and the limitations of this guideline can hinder the dissemination of the recommendations expressed. Similarly, the wide availability of recommended resources (no impact to obtain resources) and the high level of evidence (systematic review of randomized clinical trials) are facilitators for the dissemination of this guideline.

RECOMMENDATION

For patients undergoing ERCP with endoscopic biliary sphincterotomy, the use of pure cut routinely (or endocut with low effect 1 or 2) is the acceptable strategy. Mixed currents (endocut or blend) are used in cases with increased risk of bleeding or as a rescue strategy for bleeding more than expected during the procedure.

The level of evidence varies from very low to high depending on the outcome analyzed.

AUTHOR'S CONTRIBUTIONS

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

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ANNEX

Methods

Protocol and Registration

This study was carried out according to PRISMA guidelines and registered in PROSPERO (International Prospective Register of Systematic Reviews) under the record CRD42018109713[7].

Eligibility criteria

Only randomized controlled trials that compared at least two electric current modalities with the necessary data for our analysis were selected. There was no restriction on the language or publication date.

All selected studies included patients older than 18 years who underwent ERCP with biliary sphincterotomy randomized to different modes of electric current.

Studies involving patients with anatomical alterations in the gastrointestinal (GI) tract such as gastrectomy with Billroth II or Roux-en-Y reconstructions or studies involving pancreatic sphincterotomy were excluded.

Search strategy, study selection, and data collection

Initially, two authors performed the search by using title and abstract in the Medline, Embase, Central Cochrane and Lilacs databases, and grey literature. Later, the full text of the studies of interest was assessed. Disagreements were resolved after consensus with a third author. The search strategy was updated till September 2020. All prospective randomized studies that reported the outcomes of interest were included without restriction as to language, modality, or year of publication. The authors used Excel spreadsheets for data collection.

Search strategy

The following search strategy was used on Medline: (((((papillotomy OR Sphincterotomy OR Sphincterotomies OR Sphinc-terotome OR Sphincteroplasty OR Sphincteroplasties) OR ((Retrograde Cholangiopancreatography, Endoscopic OR Cholangiopancreatographies, Endoscopic Retrograde OR Endoscopic Retrograde Cholangiopancreatographies OR Ret-rograde Cholangiopancreatographies, Endoscopic OR Endo-scopic Retrograde Cholangiopancreatography OR ERCP) AND (cut OR electrosurg* OR knife OR blend OR current OR electric* OR Thermocoagulation OR Galvanocautery OR Diathermy OR Fulguration OR vio 200 OR vio 300 OR ERBEOR valley lab OR valleylab OR WEM OR blend OR current OR electrocautery OR cautery OR insulation OR insulated OR coagulation OR endocut OR waves))))))).

In the remaining databases, we used search strategies obtained from the one expressed above.

Data analysis

We used the RevMan 5 software (Review Manager version 5.3.5 – Cochrane Collaboration Copyright© 2014) for the meta-analysis and calculation of the absolute risk difference.

We included only dichotomous variables, employing the risk difference with the Mantel-Haenszel test. Statistically, we considered the 95% confidence interval (CI) and $p < 0.05$. The results were expressed as forest plots.

Heterogeneity was assessed using the Higgins test (I^2), with a fixed effect for low heterogeneity ($I^2 < 50\%$). For $I^2 > 50\%$ (high heterogeneity), we performed a sensitivity analysis using funnel plot to identify outliers. If, after excluding the outlier, $I^2 < 50\%$, the fixed effect was maintained. If, the exclusion of the outlier, $I^2 > 50\%$, the study was maintained (true heterogeneity), and the random effect was applied.

Methodology quality and risk of study bias

The risk of bias in the studies was assessed individually using the Cochrane tool[8].

The quality of the evidence (level of certainty) of each outcome was performed according to the GRADE recommendations, using the GRADEpro software[9].

Heterogeneity in the bleeding definition

Once the definition of bleeding was heterogeneous among the included studies, we made efforts to standardize it. For this, we considered mild bleeding as self-limited (that increased during sphincterotomy, however with no need for any form of intervention); moderate when there was a need for intervention during ERCP and later drop in hematimetric levels or melena; and severe cases involved clinical repercussions with the need for blood transfusion or the need for new therapeutic procedures.

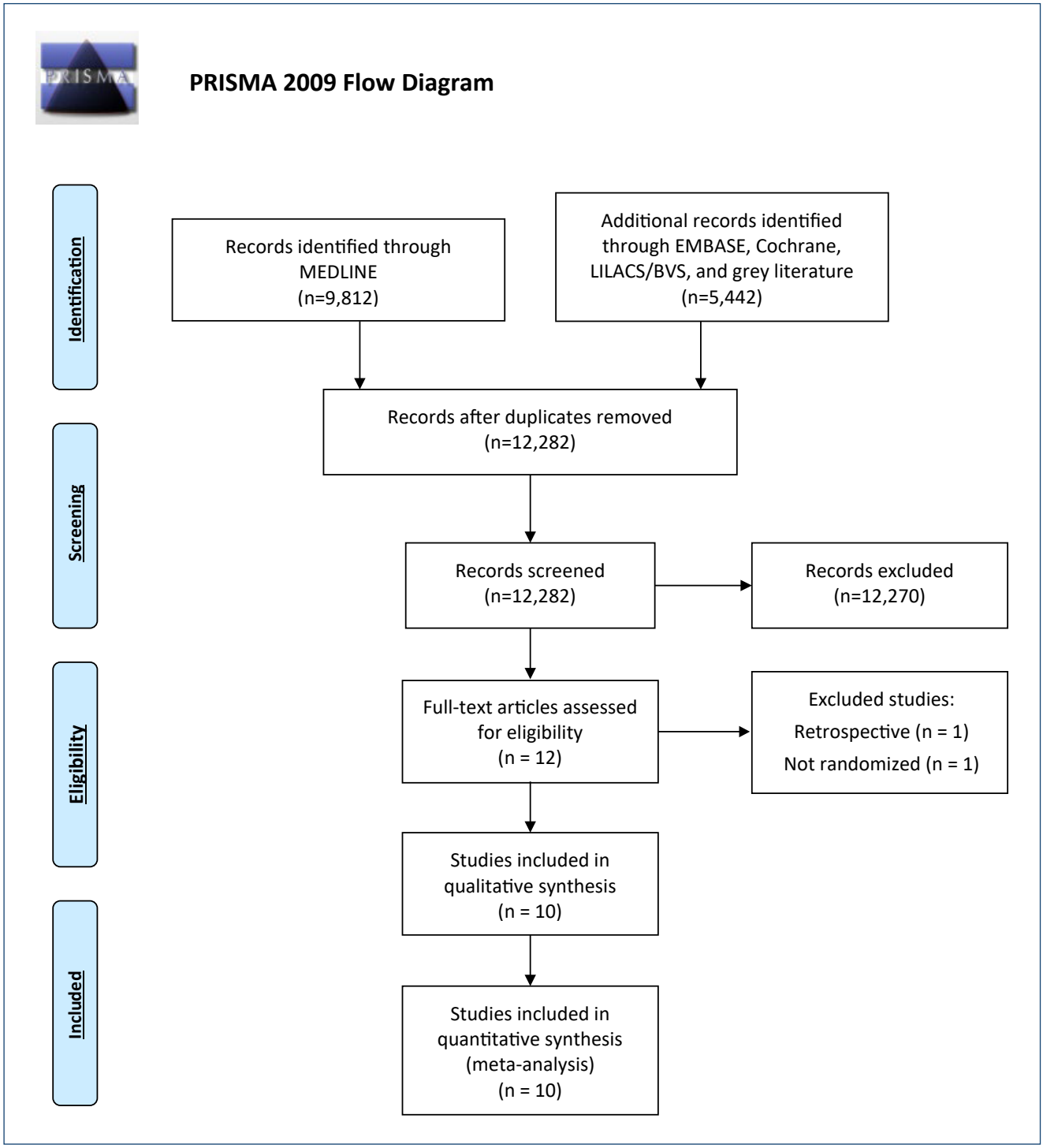

Figure 1. Flowchart of study selection according to PRISMA.

Forest plots

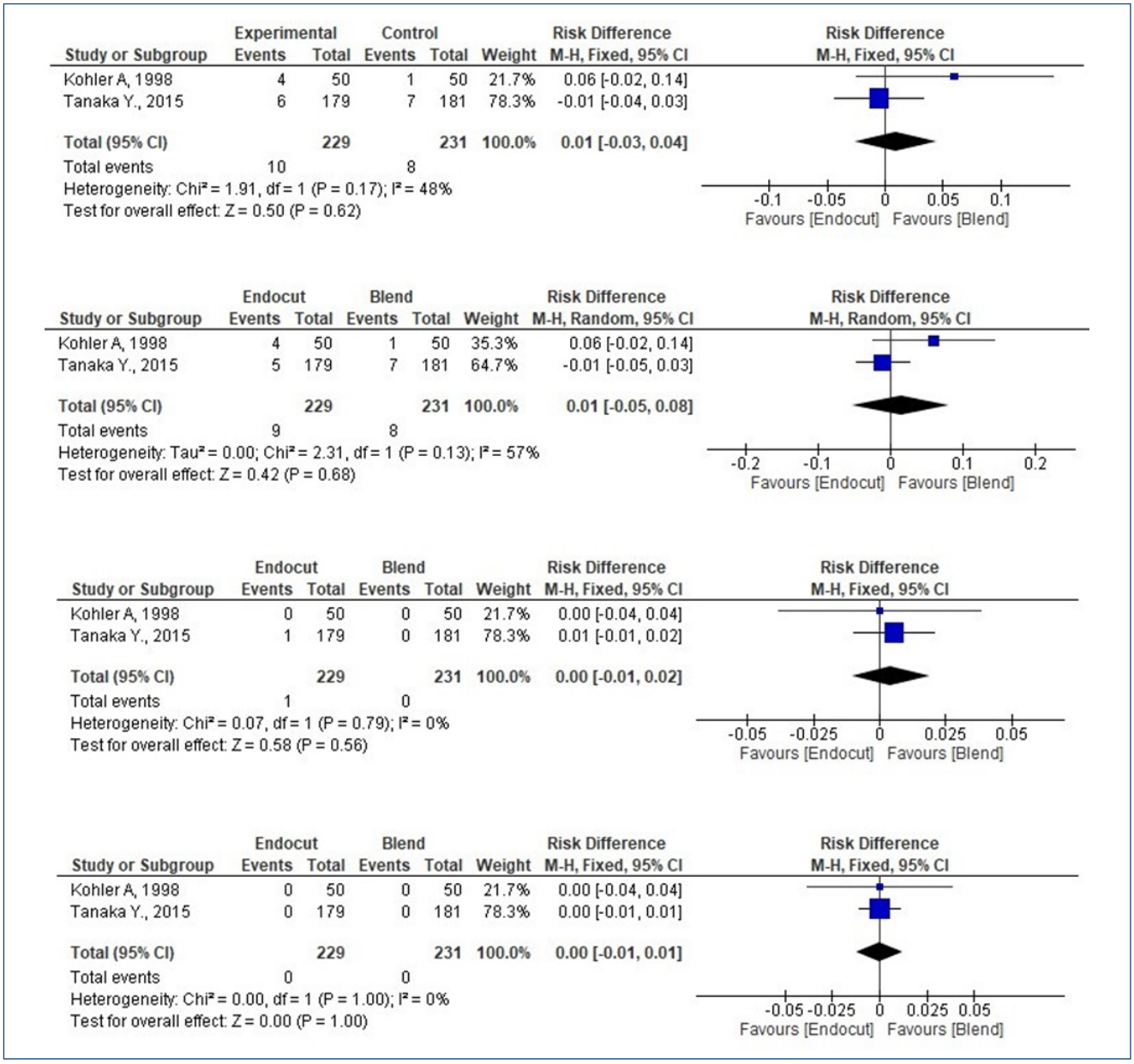

Figure. Pancreatitis in general, mild, moderate, and severe (endocut vs. blend).

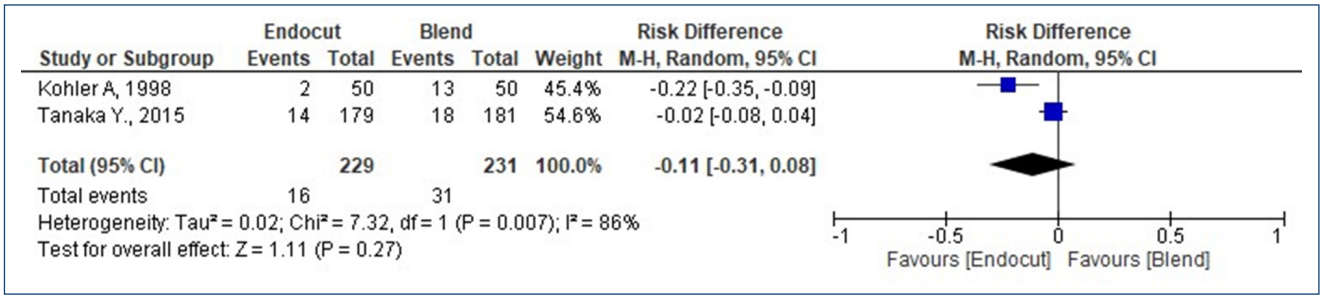

Figure. Bleeding in general (endocut vs. blend).

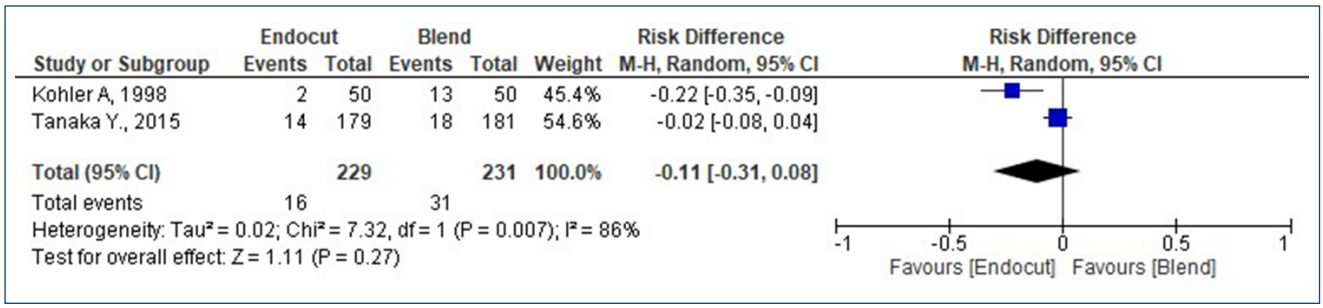

Figure. Mild bleeding (endocut vs. blend).

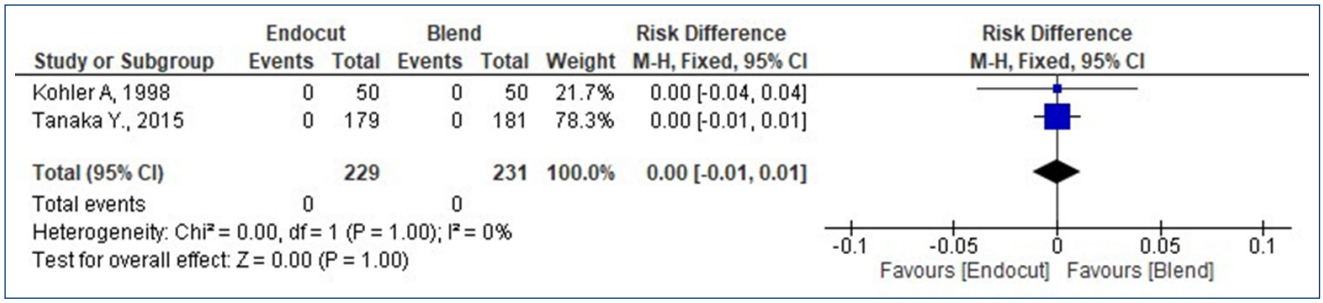

Figure. Moderate bleeding (endocut vs. blend).

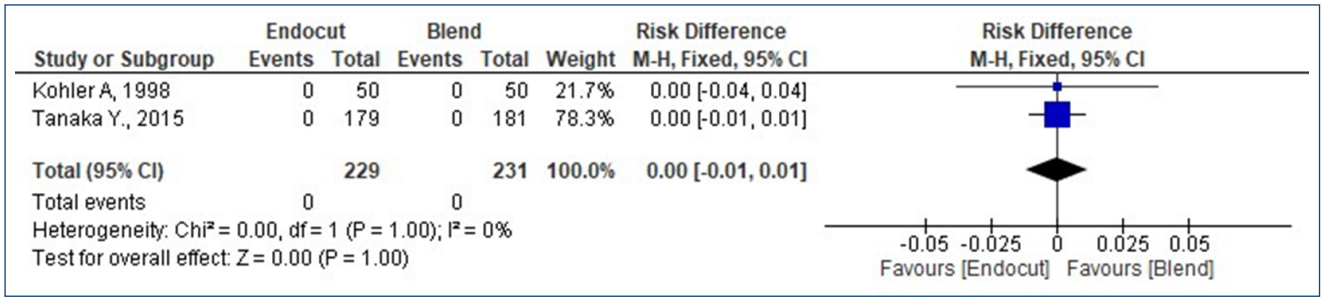

Figure. Severe bleeding (endocut vs. blend).

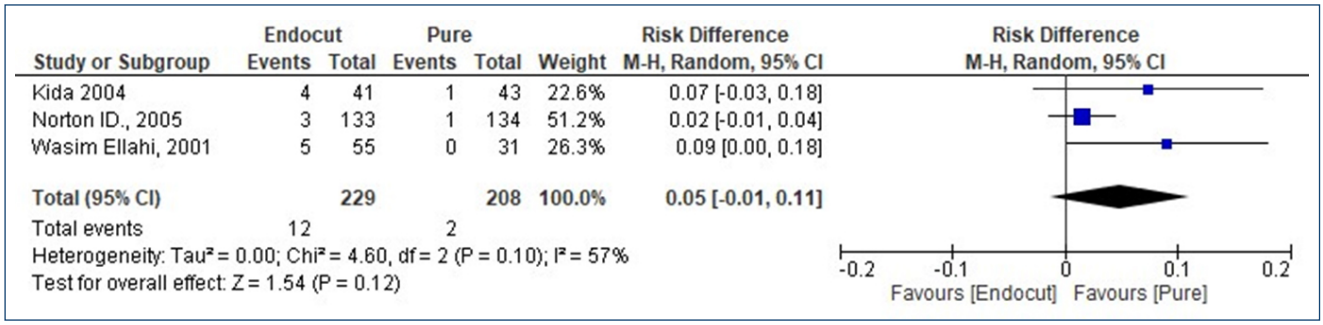

Figure. Pancreatitis in general (endocut vs. pure cut).

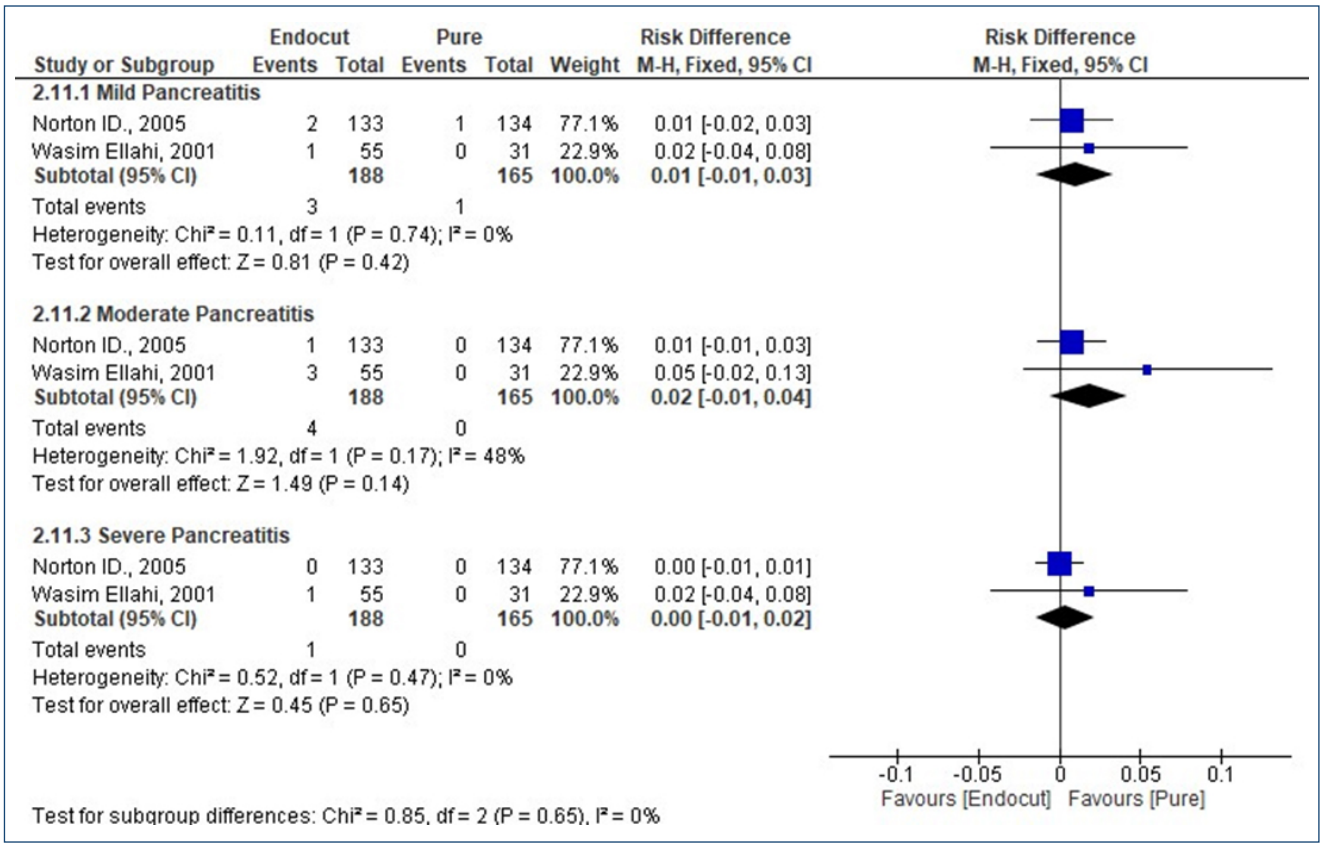

Figure. Mild, moderate, and severe pancreatitis (endocut vs. pure cut).

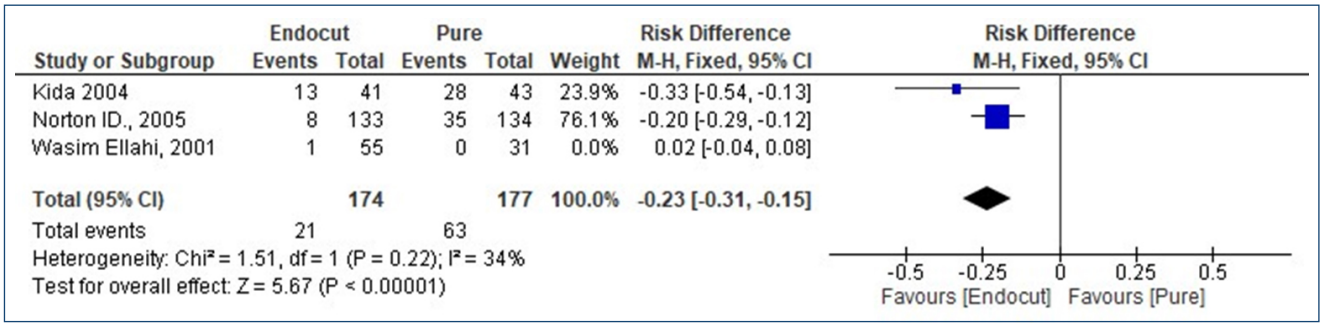

Figure. Bleeding in general (endocut vs. pure cut).

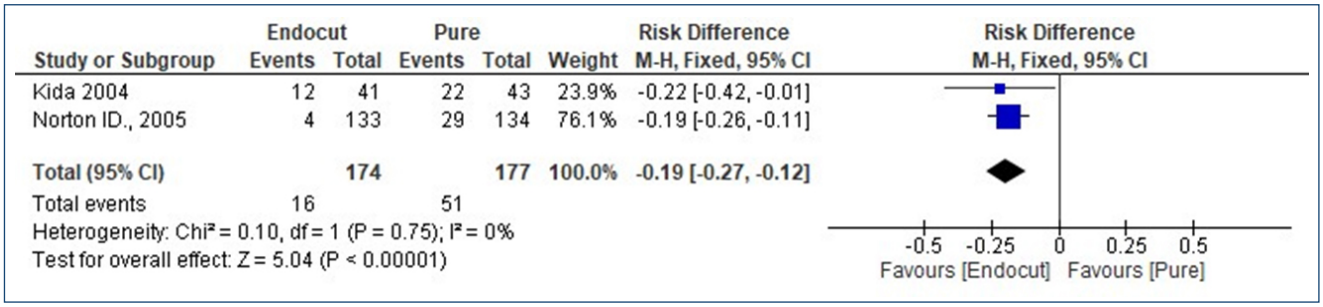

Figure. Mild bleeding (endocut vs. pure cut).

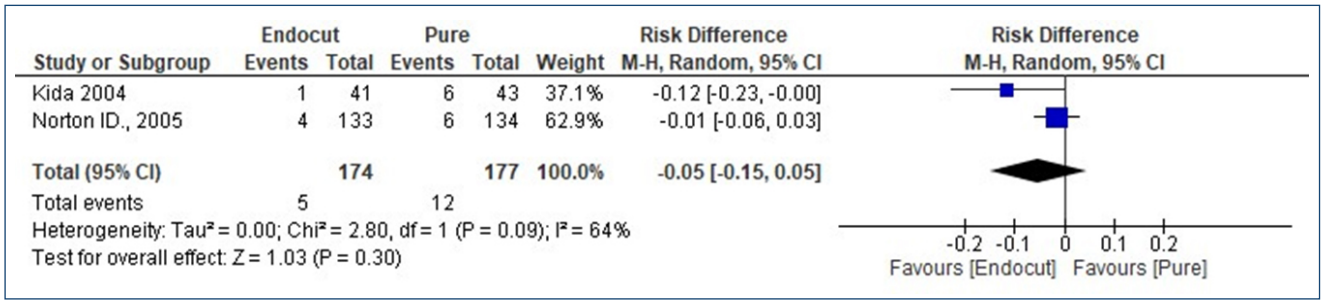

Figure. Moderate bleeding (endocut vs. pure cut).

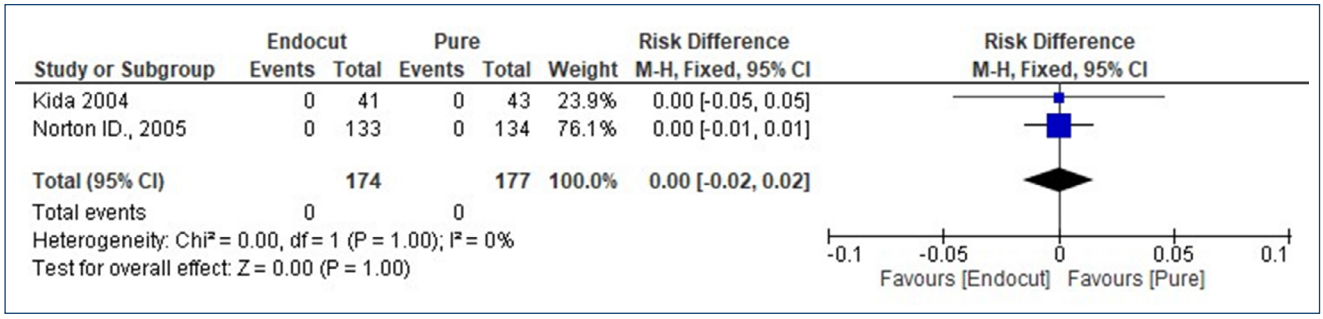

Figure. Severe bleeding (endocut vs. pure cut).

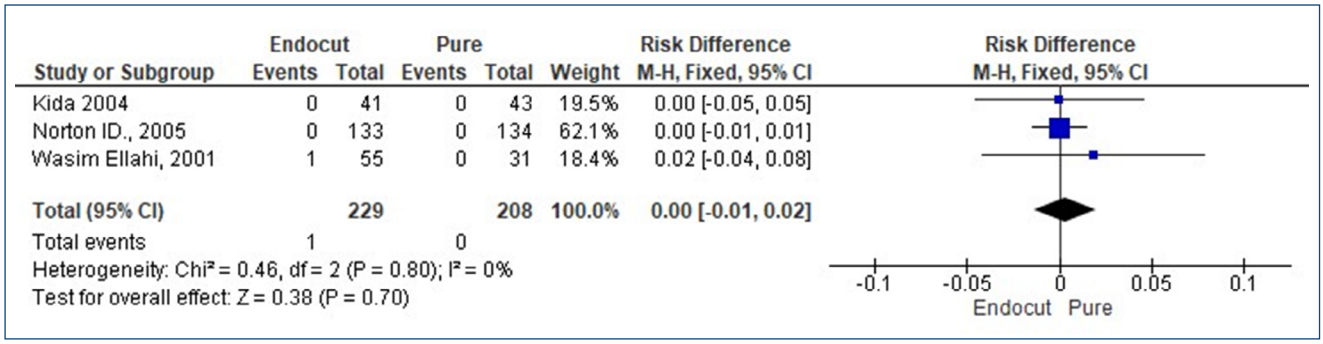

Figure. Perforation (endocut vs. pure cut).

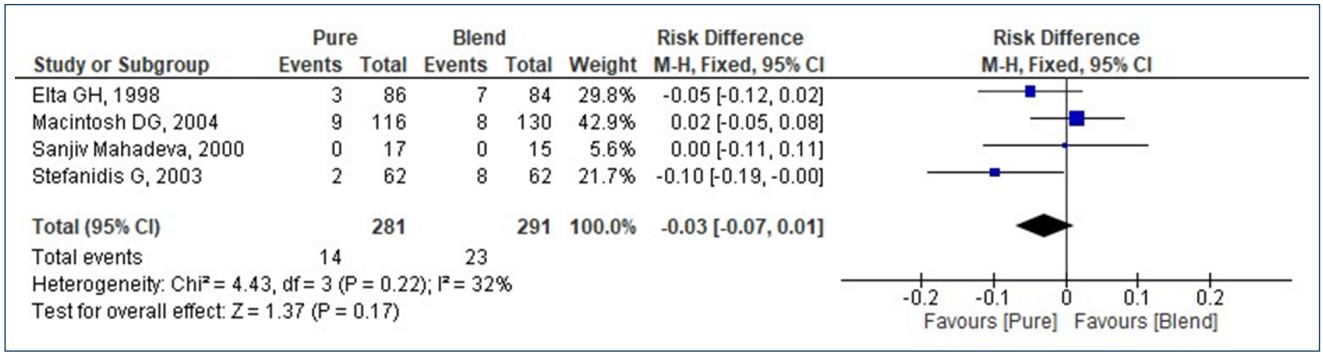

Figure. Pancreatitis in general (pure cut vs. blend).

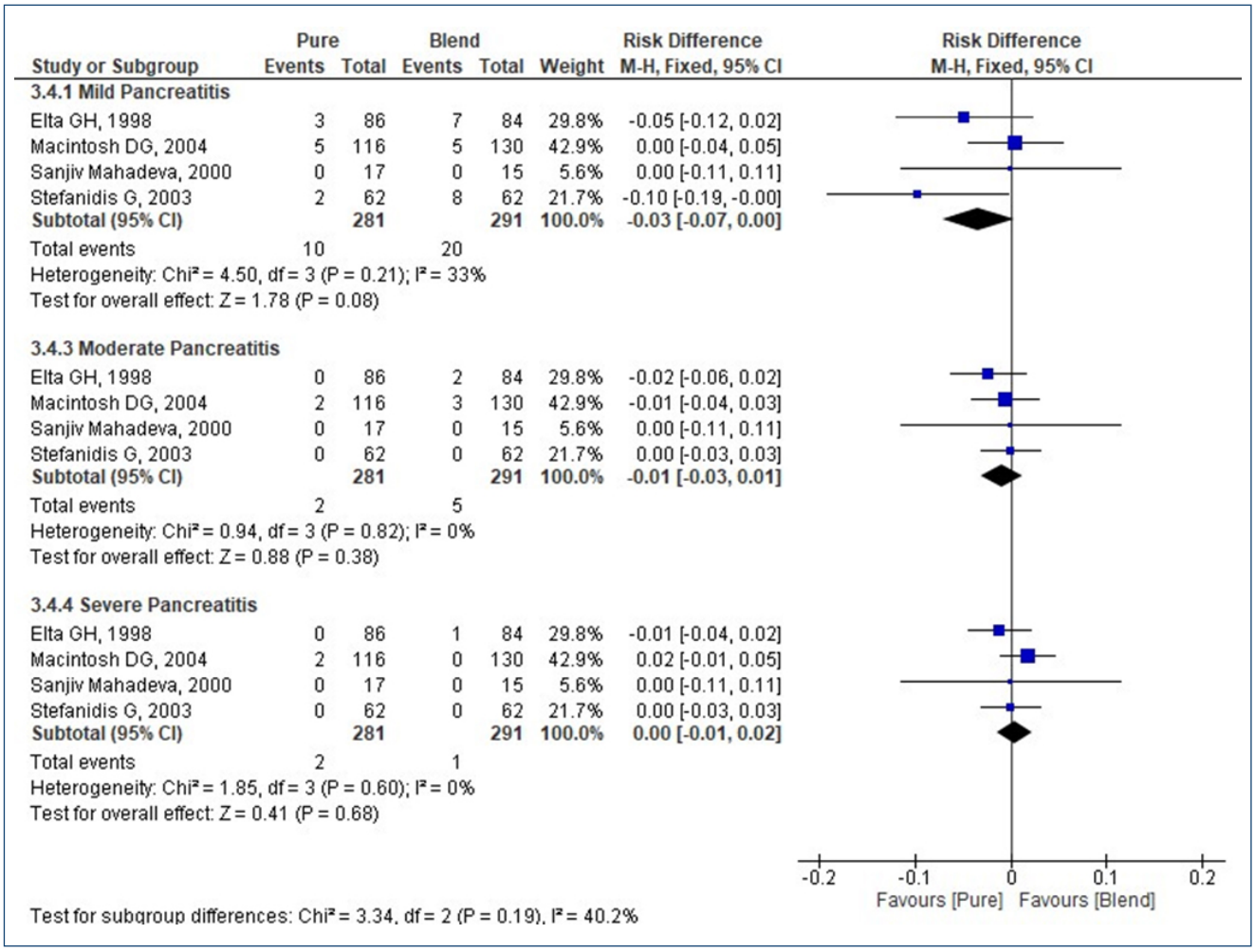

Figure. Mild, moderate, and severe pancreatitis (pure cut vs. blend).

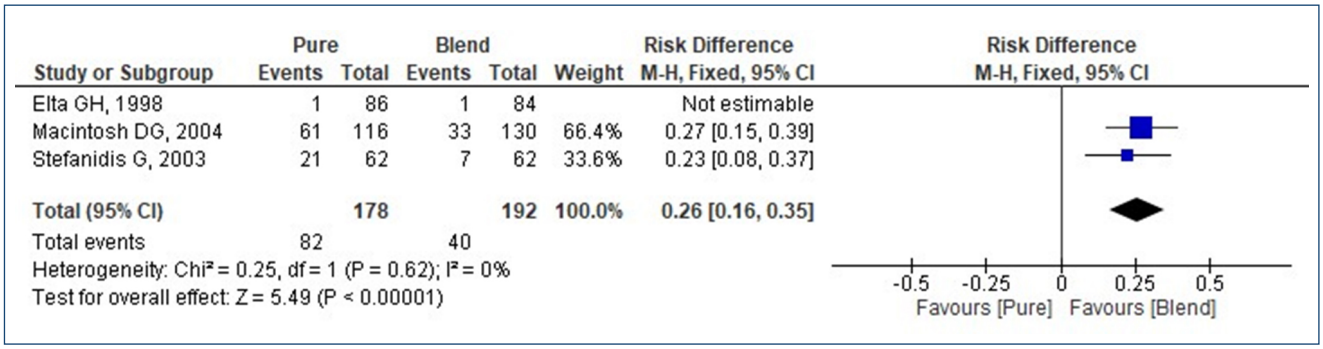

Figure. Bleeding in general (pure cut vs. blend).

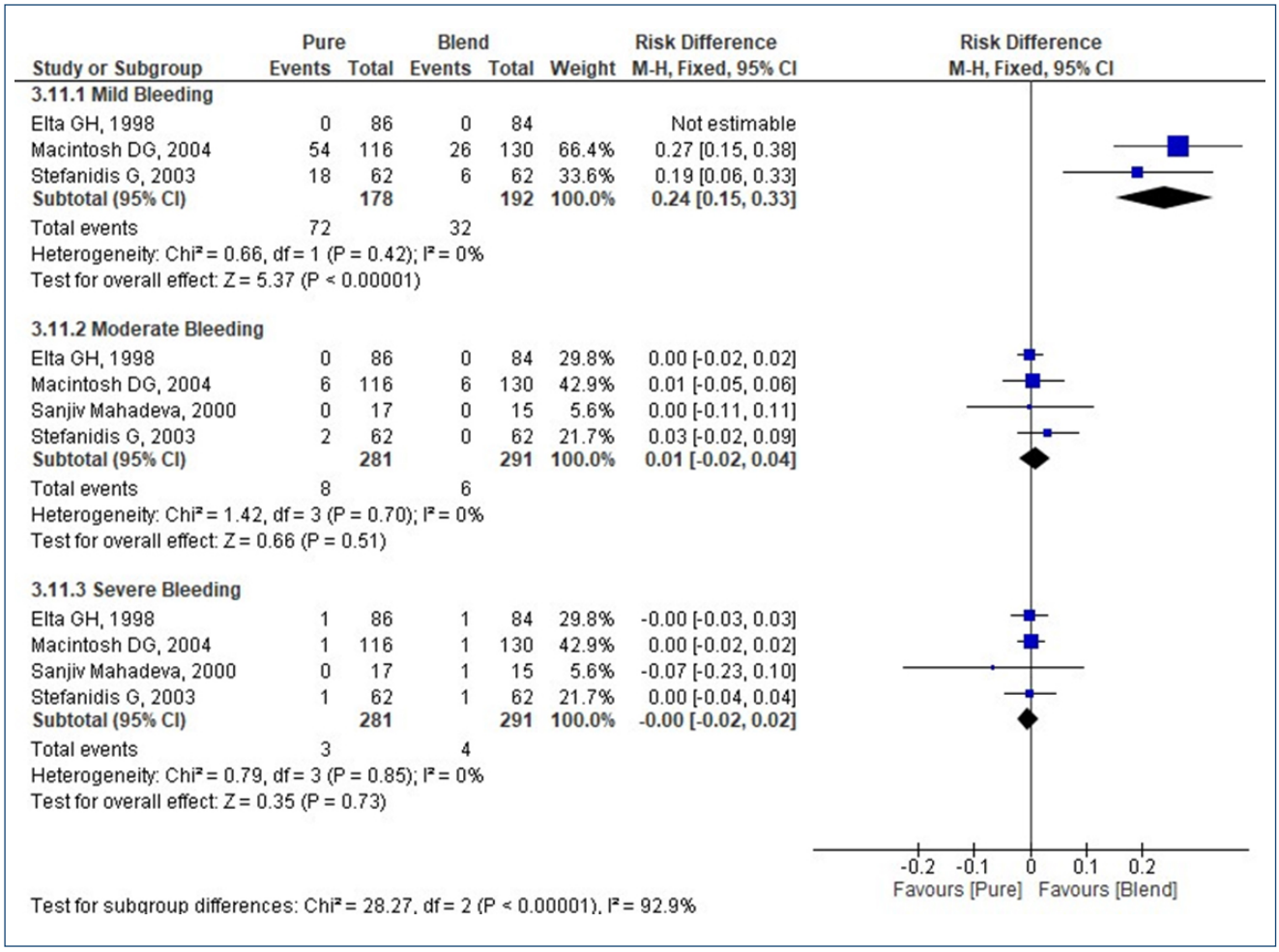

Figure. Mild, moderate, and severe bleeding (pure cut vs. blend).

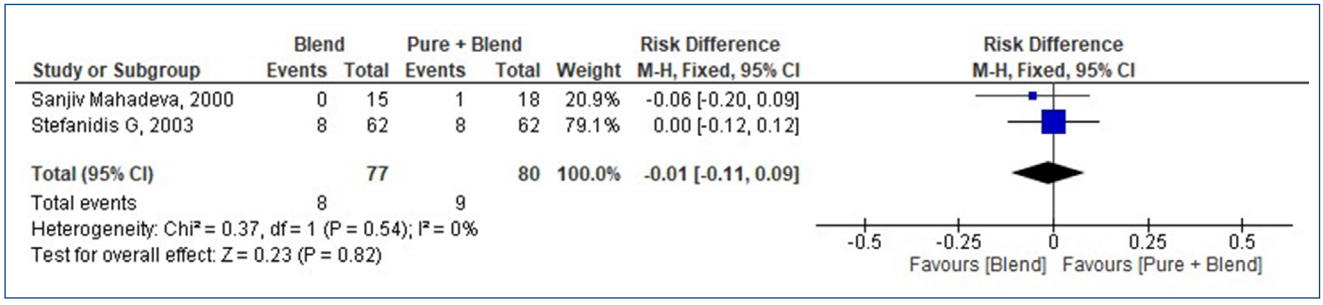

Figure. Pancreatitis in general (pure cut followed by blend vs. blend).

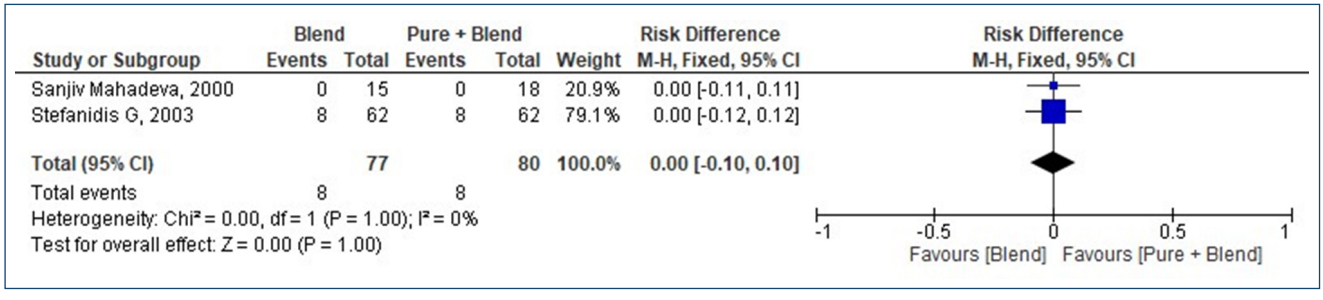

Figure. Mild pancreatitis (pure cut followed by blend vs. blend).

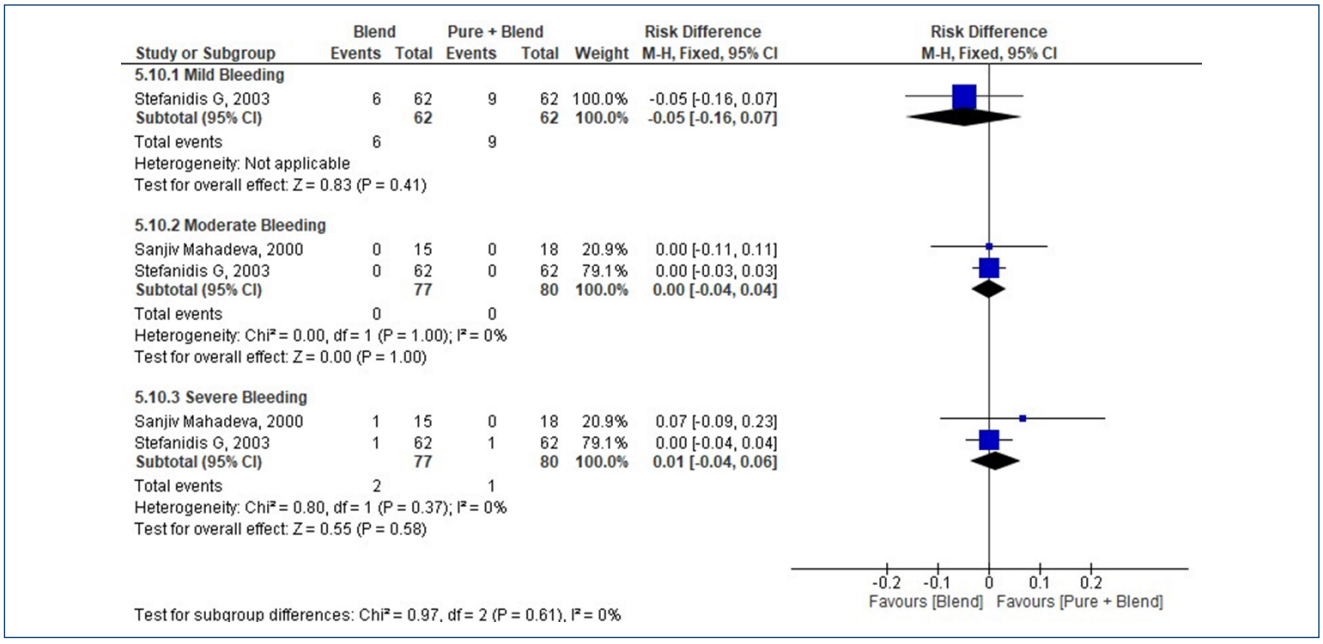

Figure. Mild, moderate, and severe bleeding (pure cut followed by blend vs. blend).

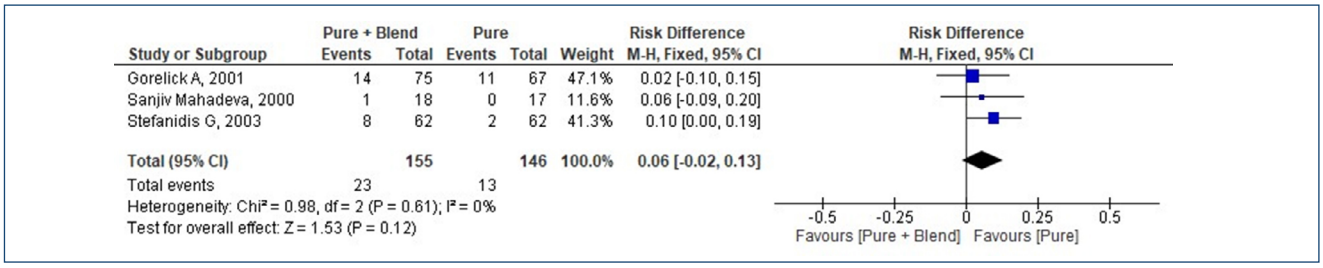

Figure. Pancreatitis in general (pure cut followed by blend vs. pure cut).

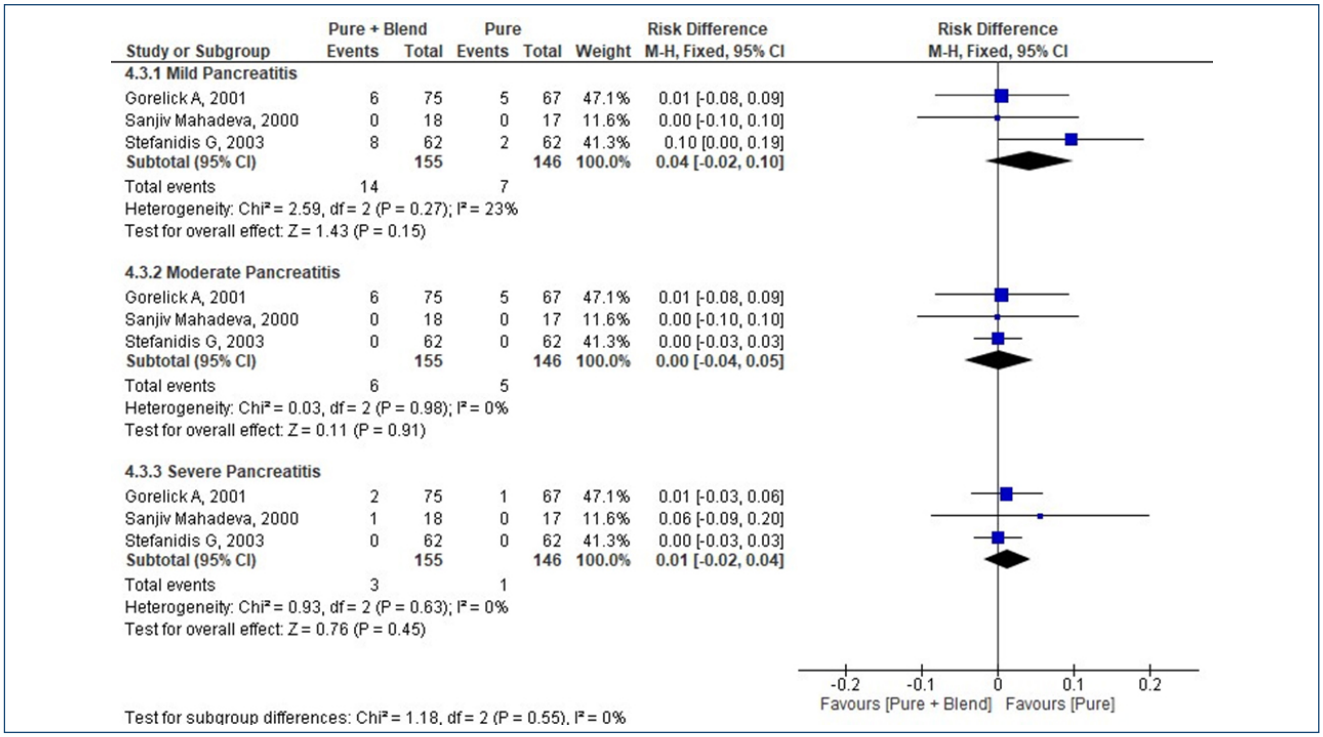

Figure. Mild, moderate, and severe pancreatitis (pure cut followed by blend vs. pure cut).

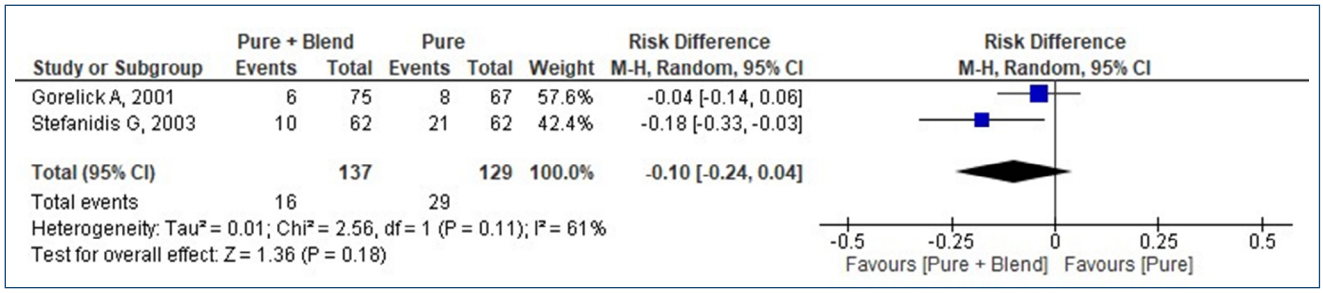

Figure. Bleeding in general (pure cut followed by blend vs. pure cut).

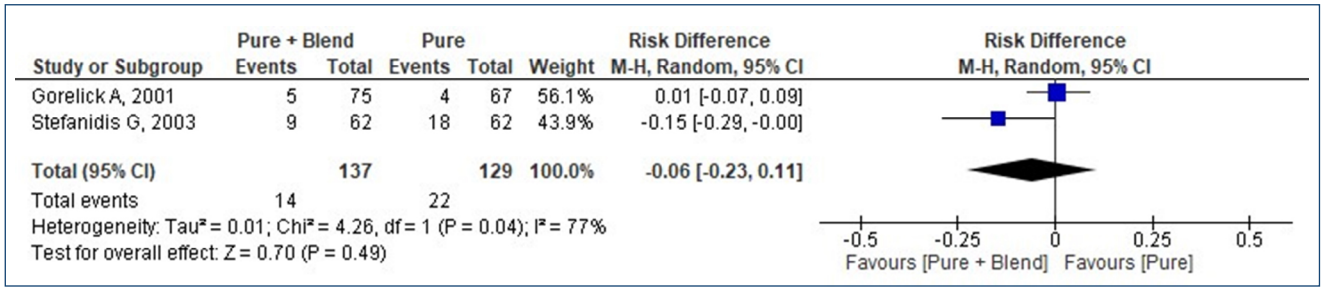

Figure. Mild bleeding (pure cut followed by blend vs. pure cut).

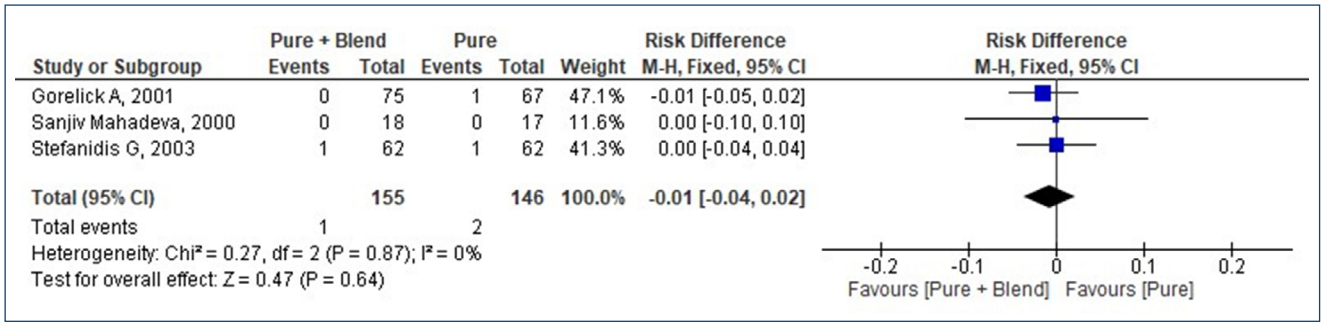

Figure. Severe bleeding (pure cut followed by blend vs. pure cut).

Grade

Evidence quality according to GRADE (endocut vs. blend).

Certainty assessment							Nº of patients		Effect		Certainty	Importance
Nº of studies	Study design	Risk of bias	Inconsistency	Indirectness	Imprecision	Other considerations	Endocut	Blend	Relative (95%CI)	Absolute (95%CI)		
Perforation												
2	Randomized trials	Not serious[a]	Not serious	Not serious	Serious[b]	None	0/229 (0.0%)	0/231 (0.0%)	Not estimable	**0 fewer per 1.000** (from 10 fewer to 10 more)	⊕⊕⊕◯ Moderate	
Pancreatitis (total)												
2	Randomized trials	Not serious[a]	Not serious	Not serious	Serious[b]	None	10/229 (4.4%)	8/231 (3.5%)	Not estimable	**10 fewer per 1.000** (from 40 fewer to 30 more)	⊕⊕⊕◯ Moderate	
Bleeding (total)												
2	Randomized trials	Very serious[a]	Very serious[c]	Not serious	Serious[b]	None	16/229 (7.0%)	31/231 (13.4%)	Not estimable	**110 more per 1.000** (from 80 fewer to 310 more)	⊕◯◯◯ Very low	

CI: confidence interval; [a] Kohler with Jadad scale <3 and a few unclear bias evaluation points. None of the studies is a double blind (difficult in endoscopy); [b]. Power < 80%; [c]. I2 > 75%.

Evidence quality according to GRADE (endocut vs. pure cut).

Certainty assessment							Nº of patients		Effect		Certainty	Importance
Nº of studies	Study design	Risk of bias	Inconsistency	Indirectness	Imprecision	Other considerations	Endocut	Pure	Relative (95%CI)	Absolute (95%CI)		
Perforation												
3	Randomized trials	Serious[a]	Not serious	Not serious	Serious[b]	None	1/229 (0.4%)	0/208 (0.0%)	Not estimable	**0 fewer per 1.000** (from 20 fewer to 10 more)	⊕⊕◯◯ Low	
Pancreatitis (total)												
3	Randomized trials	Serious[a]	Serious[c]	Not serious	Serious[b]	None	11/229 (4.8%)	2/208 (1.0%)	Not estimable	**50 fewer per 1.000** (from 120 fewer to 30 more)	⊕◯◯◯ Very low	
Bleeding (total)												
2	Randomized trials	Serious[a]	Not serious	Not serious	Not serious	None	21/174 (12.1%)	63/177 (35.6%)	Not estimable	**230 more per 1.000** (from 150 more to 310 more)	⊕⊕⊕◯ Moderate	
Cholangitis												
1	Randomized trials	Serious[d]	Not serious	Not serious	Serious[b]	None	1/55 (1.8%)	1/31 (3.2%)	Not estimable	**10 more per 1.000** (from 60 fewer to 90 more)	⊕⊕◯◯ Low	
Hyperamylasemia												
1	Randomized trials	Serious[d]	Not serious	Not serious	Serious[b]	None	12/41 (29.3%)	5/43 (11.6%)	Not estimable	**180 fewer per 1.000** (from 350 fewer to 10 fewer)	⊕⊕◯◯ Low	

CI: confidence interval; [a] All studies included with Jadad scale < 3. A few unclear bias evaluation points in Kida and Wasim; [b] Power < 80%; [c] 50–75%; [d] Jadad scale < 3. A few unclear bias evaluation points.

Evidence quality according to GRADE (pure cut vs. blend).

Certainty assessment							Nº of patients		Effect		Certainty	Importance
Nº of studies	Study design	Risk of bias	Inconsistency	Indirectness	Imprecision	Other considerations	Pure	Blend	Relative (95%CI)	Absolute (95%CI)		
Cholangitis												
1	Randomized trials	Serious[a]	Not serious	Not serious	Serious[b]	None	0/86 (0.0%)	1/84 (1.2%)	Not estimable	**10 more per 1.000** (from 20 fewer to 40 more)	⊕⊕◯◯ Low	
Pancreatitis (total)												
4	Randomized trials	Serious[c]	Not serious	Not serious	Serious[b]	None	14/281 (5.0%)	23/291 (7.9%)	Not estimable	**30 more per 1.000** (from 10 fewer to 70 more)	⊕⊕◯◯ Low	
Bleeding (total)												
2	Randomized trials	Not serious[d]	Not serious	Not serious	Not serious[b]	None	82/178 (46.1%)	40/192 (20.8%)	Not estimable	**260 fewer per 1.000** (from 350 fewer to 160 fewer)	⊕⊕⊕⊕ High	

CI: confidence interval; [a] Jadad scale < 3. Inappropriate randomization method; [b]Power < 80; [c] Jadad scale < 3 in two studies. Only one (out of four) double blind. A few unclear bias evaluation points in the studies; [d] Stefanidis with Jadad scale < 3 and no sample size. Also not a double blind.

Evidence quality according to GRADE (pure cut followed by blend vs. blend).

Certainty assessment							Nº of patients		Effect		Certainty	Importance
Nº of studies	Study design	Risk of bias	Inconsistency	Indirectness	Imprecision	Other considerations	Pure + Blend	Pure	Relative (95%CI)	Absolute (95%CI)		
Pancreatitis (total)												
3	Randomized trials	Serious[a]	Not serious	Not serious	Serious[b]	None	23/155 (14.8%)	13/146 (8.9%)	Not estimable	**60 fewer per 1.000** (from 130 fewer to 20 more)	⊕⊕◯◯ Low	
Bleeding (total)												
2	Randomized trials	Not serious[a]	Serious[c]	Not serious	Serious[b]	None	16/137 (11.7%)	29/129 (22.5%)	Not estimable	**100 more per 1.000** (from 40 fewer to 240 more)	⊕⊕◯◯ Low	

CI: confidence interval; [a] Stefanidis with Jadad scale < 3 and no sample size. A few unclear bias evaluation points in the articles. None of the articles is a double blind (difficult in endoscopy); [b] Power < 80; [c] 50–75%.

Evidence quality according to GRADE (pure cut followed by blend vs. pure cut).

Certainty assessment							Nº of patients		Effect		Certainty	Importance
Nº of studies	Study design	Risk of bias	Inconsistency	Indirectness	Imprecision	Other considerations	Blend	Pure + Blend	Relative (95%CI)	Absolute (95%CI)		
Pancreatitis (total)												
2	Randomized trials	Serious[a]	Not serious	Not serious	Serious[b]	None	8/77 (10.4%)	9/80 (11.3%)	Not estimable	**10 more per 1.000** (from 90 fewer to 110 more)	⊕⊕◯◯ Low	
Bleeding (total)												
1	Randomized trials	Not serious[c]	Not serious	Not serious	Serious[b]	None	7/62 (11.3%)	10/62 (16.1%)	Not estimable	**50 more per 1.000** (from 50 more to 50 more)	⊕⊕⊕◯ Moderate	

CI: confidence interval; [a] Stefanidis with Jadad scale < 3 and no sample size. A few unclear bias evaluation points in Sanjiv. None of the studies is a double blind; [b] Power < 80; [c] Jadad scale < 3, not a double blind and no sample size.

Table 1. Summary of the risk of bias in the included studies.

Study	Cochrane risk of bias tool								
	Rsg	Acs	Bpp	Boa	Iod	O	Pf	Itt	Ss
Tanaka[12]	+	+	−	+	+	+	+	+	+
Norton[14]	−	?	−	−	+	+	+	+	+
Kida[11]	?	?	?	?	+	+	+	+	?
Macintosh[13]	+	+	+	+	+	+	+	+	+
Stefanidis[16]	+	+	−	+	+	+	+	+	−
Wasim[18]	?	?	?	?	+	+	?	+	?
Gorelick[17]	+	?	−	+	+	+	+	+	?
Mahadeva[15]	?	?	−	+	+	+	−	−	?
Kohler[4]	?	?	?	?	+	+	+	+	?
Elta[10]	−	?	−	+	+	+	+	+	?

Low risk of bias Unclear risk of bias High risk of bias

Rsg: random sequence generation (selection bias); Acs: allocation concealment (selection bias); Bpp: blinding participants and personnel (performance bias); Boa: blinding of outcome assessment (detection bias); Iod: incomplete outcome data (attrition bias); O: outcome; Pf: prognostic factor; Itt: intention to treat analysis; Ss: sample size

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The Pulmonary Metastasectomy in Colorectal Cancer study calls for reconsideration of the clinical effectiveness of this widespread practice

Tom Treasure[1*], Fergus Macbeth[2], Riad Younes[3]

Dear Editor,

We read with great interest the report by Dr. Oya Yildiz and colleagues on pulmonary metastasectomy for patients with colorectal cancer (CRC)[1]. The authors make brief reference to the preliminary results of the Pulmonary Metastasectomy in Colorectal Cancer (PulMiCC) trial[2], which drew our attention to their report. The randomized controlled trial (RCT) was nested within a cohort study of 512 patients, which has now been reported in full as has the completed RCT[3,4]. We believe that the findings of the full PulMiCC study are directly relevant to the interpretation of their findings.

While the PulMiCC study was ongoing, the authors worked together in the Surgical and Interventional Trials Unit (SITU) at University College London on a meta-analysis of monitoring protocols following resection of primary CRC[5]. There were 16 randomized trials, of which 11 provided data suitable for meta-analysis. The purpose of these monitoring protocols is to detect relapse, and particularly metastatic disease, with the intention of increasing the numbers of patients suitable for metastasectomy. They were successful in advancing the diagnosis by a median of 10 months (IQR 5–24), but there was no survival gain. A Cochrane meta-analysis provided similar conclusions[6].

The meta-analysis raised doubts about the assumed survival benefit of CRC lung metastasectomy, which has become the standard of care internationally. It was said to be "a pillar of modern thoracic surgery" in an Editorial in the *European Journal of Cardio-Thoracic Surgery* (*EJCTS*)[7]. Two observational studies of metastatic CRC (mCRC) were cited with a pooled 5-year survival of 60%. And then, in the United States, the Society of Thoracic Surgeons (STS) in an Expert Consensus Document reported that "survival is assumed to be zero" in patients with lung metastases[8]. The gap between these figures — 60% versus zero — was considered to be the effect attributable to lung metastasectomy.

In the full PulMiCC study, 512 patients gave informed consent to be considered for lung metastasectomy and baseline data were collected according to RCT standards. Of them, 28 were excluded because — during initial evaluation — their nodules were found to not be CRC metastases. Because of the widely held belief in the 60% increase in 5-year survival, the clinicians' stated equipoise was challenged and only 93 patients were randomized. Of the remainder, 263 underwent metastasectomy and 128 were not operated, comprising 391 patients in the nonrandomized cohort. The survival of these two groups is illustrated in the Kaplan-Meier analysis in the upper panel of the (Figure 1). The operated patients had a survival of about 60% in line with the best reported series cited in the *EJCTS* editorial[7]. But the survival of patients selected to *not* have an operation was not zero contrary to the STS consensus[8].

The data collected at baseline under trial conditions permitted a full analysis of the features of the patients in the electively operated and unoperated groups. These data were of course used in making these elective decisions. The operated group was dominated by solitary metastases (69% vs. 35%) and far fewer had more than five metastases (0.8% vs. 10.3%). There were also fewer patients with hepatic involvement (28% vs. 36%) and fewer with elevated carcinoembryonic antigen levels (12% vs. 20%). They were younger (60 vs. 67 years), more had unimpaired performance (68% vs. 36% using Eastern Co-operative Oncology Group scores), and they had better lung function (predicted FEV_1 96% vs. 87%). All of these factors favored

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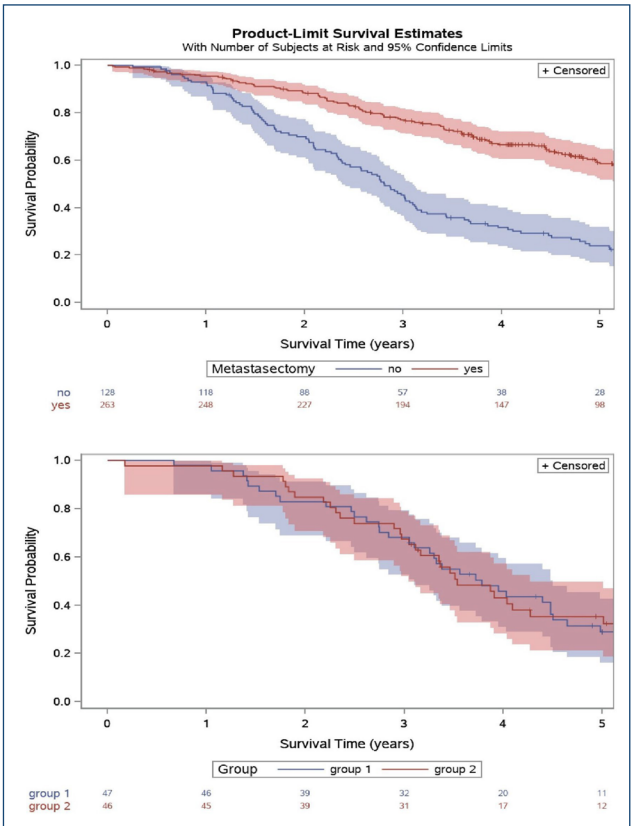

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Figure 1. Five-year survival estimates of patients at risk.

better survival in patients having metastasectomy irrespective of subsequent treatment[4].

To know how much of a difference was actually attributable to lung metastasectomy required randomized control data, shown in the lower panel of the figures. These factors were excellently balanced in the two arms of the randomized trial. The two curves were weaving in and out of each other. The median survival was longer in the control group (45.6 months vs. 42.0 months), but there was no difference in survival at any time point. Estimating the possible difference in survival at 5 years is precluded by the broad confidence bands around the arms of 46 and 47 patients, but it cannot be anything like the magnitude believed. It is also important to know that in the RCT, the performance status diminished at a similar rate in the two groups, there was no psychological benefit, and that there was a relative loss of lung function in the operated patients. There was no benefit but demonstrable harm.

An inescapable feature of the clinical care of these patients is that time elapses between when they are first identified and when the operation is actually carried out. Patients who progress during this time, either in the lung or at other sites, are less likely to have an operation and so those eventually selected are unlikely to die in the next year or so. This introduces guarantee time bias[9]. A "guarantee time" also applies in RCTs, but it affects both arms. Provided the report is on intention to treat and from time of randomization (as it should be), this eliminates the bias.

The report of Yildiz et al. is exceptional in that they provide information about the denominator from which their patients were drawn in contrast to the publications in the systematic review[10]. Yildiz et al. reported that 33 patients who had lung metastasectomy were among 607 patients treated for mCRC in their center (5.4%, 95% confidence intervals 3.8–7.6%). From English National Health Service data (2005–2013), we estimate that 4.9% of about 70,000 patients with mCRC had pulmonary metastasectomy, a very similar figure. The degree of case selection is closely comparable.

The PulMiCC cohort also provides data on further treatments[11]. Yildiz et al. draw the following conclusion from their experience: "Therefore, we have to make a vigilant follow-up for the second lung relapse to seize an opportunity for the second metastasectomy[1]." The PulMiCC analysis of additional treatments cannot refute the belief that there is benefit from repeat metastasectomy because there was no controlled comparison, but on statistical review of the claims, it seems unlikely[12,13]. Also reported from PulMiCC are the quality of life, health utility, and the burden of additional treatments[3,11,14]. Given the low likelihood of survival benefit in the PulMiCC RCT[3], it seems difficult to justify these treatment burdens.

Yildiz and colleagues are very realistic about the limitations of their small study.

The surgical treatment of metastatic disease has grown, and belief in its effectiveness is sustained by expert case selection of those naturally most likely to survive. This is compounded by guarantee time bias, confusing association with causation, affirmation bias, and remarkable optimism. It is perhaps time for realism and a more careful appraisal of the evidence, which currently does not support the belief in a substantial survival benefit. Recent reports, including rebuttals of the PulMiCC RCT findings, suggest that the new objective of treatment is local control rather than "cure," but this is "moving the goal posts." Few patients experience symptoms from isolated lung metastases and so local control is not an important clinical issue. For systemic treatments, it is accepted that no drug should be introduced without RCT evidence and the same should be true for local interventions[15]. Sufficiently large, collaborative, and independently monitored controlled trials are needed. PulMiCC illustrates the difficulties encountered but also shows an approach to planning such trials[16].

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Comment on "Nuances between sedentary behavior and physical inactivity: cardiometabolic effects and cardiovascular risk"

Yangfen Zhang[1], Meilin Ma[1], Lianping He[1*]

Dear Editor,

We were happy to read the ingenious article by Melo et al.[1] entitled "Nuances between sedentary behavior and physical inactivity: cardiometabolic effects and cardiovascular risk," in which they illustrated some nuances between the cardiometabolic effects and the cardiovascular risk of physical inactivity and sedentary behavior. The article provides a valuable insight into different mechanisms of action between physical inactivity and sedentary behavior in cardiometabolic effects and cardiovascular risk. However, from our point of view, there are some problems that need further discussion.

The major problem of this article is that the authors take the metabolic equivalent of task (MET) as the main indicator to distinguish between sedentary behavior and physical inactivity. The authors define the MET value £1.5 as the sedentary behavior and make detailed description when the MET value >1.5. The authors point out that while sedentary, physical activities such as watching TV or using a computer can also be performed. In fact, it is not rigorous to use MET alone as an indicator to distinguish between sedentary behavior and physical inactivity because the main oxygen-consuming organ of human is brain[2]. When brain activity is intensified or tense, oxygen consumption will rise sharply, and it will also bring about increase in MET value. The accurate definition of physical inactivity in this article could be that the body is in a static state, stops all mental activities, and uses a sitting or lying position to keep the body operating at a low oxygen consumption level.

Taking it a step further, the authors' description of physical inactivity is not detailed enough. It is unscientific to distinguish between physical inactivity and physical activity only by the weekly exercise time, as exercise intensity should also be taken into account. For the evaluation of exercise intensity, we recommend the use of heart rate[3,4] when it reaches 120–180 beats per minute. And the frequency of exercise should be guaranteed to be three times a week, where each lasting for 30 min can be considered physical activity. Of course, these indicators need to be further explored by the authors to apply to people of different ages and genders.

Another problem is that the authors use a secondary analysis, but they fail to give the evaluation indicators, i.e., inclusion and exclusion criteria of the references. We suggest that the authors improve the relevant work for readers' reference.

There is another problem worthy of further discussion in this article. Although the authors stratify the age of the subjects as 5–17, 18–64, and 65 years or older, the span of age stratification between 18 and 64 is too large, which may lead to residual confounding. Because human body functions would be weakened with age[5], accompanied by the increasing influence of inflammatory factors, this brings challenges to distinguish the effects of age factors and sedentary behaviors and lack of physical activity on the body. Therefore, we recommend that the authors change and further refine the age of the subjects in order to obtain more accurate and convincing conclusion.

AUTHORS' CONTRIBUTIONS

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

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Comment on "Effect of the Mediterranean diet in patients with chronic spontaneous urticaria"

Junxiang Jiang[1], Meilin Ma[1], Lianping He[1*]

Dear Editor,

We were delighted to read the article by Ayvaz HH[1]. The article established that the Mediterranean diet was effective in suppressing the symptoms of chronic idiopathic urticaria. This may open up new possibilities for the treatment of chronic spontaneous urticaria (CSU). The authors used multivariate analysis to find that the Mediterranean diet score (MDS), HDL levels, and depression were significantly related to CSU. It also broadens the thinking for the treatment of CSU. However, in our point of view, there are still some issues that need further discussion.

The average age of the patient group was 38.6±13.0 years (18–65 years), and the average age of the control group was 38.7±13.8 years (p=0.983). Therefore, the authors did not conduct experiments on children and adolescents under the age of 18 years. Due to tight schoolwork and high learning pressure, students are more likely to have emotional eating behaviors[2]. Moreover, presenting restrained eating behaviors is statistically associated with older age and higher adherence to the Mediterranean diet. In addition, gender[3] is also a factor that affects CSU. The neutral ratio of the experimental subjects is not coordinated, which may also affect the reliability of the results. Therefore, we recommend that the authors can adjust the male-to-female ratio and age span of the experimental subjects so that the results and conclusions may be more convincing.

Furthermore, in this article, the authors used the urticaria symptom activity score over 7 days (UAS7) to measure the severity of chronic idiopathic urticaria. However, UAS7 does not clearly indicate the overall or average severity of itching during the evaluation period[4]. In addition, patients' quantitative itching was subacute and inconclusive. The itch severity and hive counts may change rapidly. Therefore, we suggest that the authors can provide quantitative data, such as the proportion of differentially expressed transcripts between disease and non-disease at baseline. This way the authors can improve the reliability and validity of the conclusion.

Additionally, the Mediterranean diet is widely used in the prevention and treatment of chronic inflammatory diseases. Many experts have explained and introduced this diet in detail, and some exercise patterns may be used to reflect its effects. Skeletal muscle mass and strength are positively correlated with Mediterranean diet patterns[5]. Among the patients surveyed, diet and exercise are likely to be related to each other. For example, some patients may rarely exercise, while others may insist on exercising and having a strong body. Their skeletal muscle content may affect the results of the experiment. However, the authors did not mention the influence of exercise on the results of this research and did not exclude its influence in the experiment.

AUTHORS' CONTRIBUTIONS

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

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Doxofylline in acute exacerbation of chronic obstructive pulmonary disease

Omer Erdem Sevik[1*], Mustafa Emin Canakci[1]

Dear Editor,

We have read with great interest the article entitled "Efficacy and safety of combined doxofylline and salbutamol in treatment of acute exacerbation of chronic obstructive pulmonary disease" by Xianrong Du et al.[1]. We thank the authors for their study on a condition that has high morbidity and mortality and is one of the major reasons for admission to the emergency department worldwide[2,3]. However, we would like to make some remarks.

Methylxanthines were used in acute exacerbation of chronic obstructive lung diseases (AECOPD) for many years. But many studies revealed that methylxanthines play no role in AECOPD. There are continuous drawbacks on its use because of its side effects such as nausea, tremors, and dysrhythmias[4]. In the introduction of this article, the authors state that methylxanthines are commonly used as bronchodilators, but current guidelines do not suggest the use of methylxanthines for the treatment of AECOPD[3,4].

Inclusion criteria state that patients who were younger than 80 years were included in the study. There is no clear base for this distinction. Even if there is, the authors did not explain the reason behind it. We have failed to understand why patients who were older than 80 years were not included in the study.

This study mainly investigates the time needed for symptom relief, improvement in pulmonary function indexes, and serological indicators, and there are no data on arrhythmias or heart rate. Thus, patients' vital parameters on admission and after both treatments are needed to make a deduction. The difference in the percentage, but not the statistical significance, of adverse reactions between the two groups could be the result of, as authors have stated, a relatively small study group (n=68). However, the difference in adverse reactions might be clinically significant.

However, statistically significant shorter remission times of symptoms, improvement in pulmonary function indexes, and serological indicators show the efficacy of combined treatment, but salbutamol is a standard therapy according to the current guidelines[4]. It might be more proper to compare doxofylline as opposed to salbutamol, which this study has taken as the experimental group.

AUTHORS' CONTRIBUTIONS

OES: writing – original draft. **MEC:** writing – review & editing.

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"Similitude in Modern Pharmacology": two decades of studies contributing to the scientific basis of the homeopathic healing principle

Marcus Zulian Teixeira[1*]

Dear Editors,

Homeopathy is based on the following four scientific pillars[1]: principle of cure by similars, proving of medicinal substances on healthy individuals, use of dynamized doses, and prescription of individualized medicines. Although great importance was attributed to dynamized doses (ultra-high dilutions), the first two pillars are the fundamental premises of the homeopathic epistemological model, remaining to individualized medicine the essential condition to awakening the therapeutic response.

In the systematization of the homeopathic method of treatment, Samuel Hahnemann based the "principle of cure by similars" (principle of therapeutic similitude) on the careful observation of the effects of medicines on human health. In the essay[2] that inaugurated homeopathy in 1796 and in paragraphs 59 and 65 of the *Organon of Medicine*[3], he describes the pharmacological effects of dozens of palliative drugs of his time, discriminating the "direct primary action of drug" and the consequent and opposite "indirect secondary action of the body," evidencing the new principle of cure proposed:

> [...] Excessive vivacity follows the use of strong coffee (primary action), but sluggishness and drowsiness remain for a long time afterwards (reaction, secondary action), if this be not always again removed for a short time by imbibing fresh supplies of coffee (palliative). After the profound stupefied sleep caused by opium (primary action), the following night will be all the more sleepless (reaction, secondary action). After the constipation produced by opium (primary action), diarrhea ensues (secondary action); and after purgation with medicines that irritate the bowels, constipation of several days' duration ensues (secondary action). And in like manner it always happens, after the primary action of a medicine that produces in large doses a great change in the health of a healthy person, that its exact opposite, when, as has been observed, there is actually such a thing, is produced in the secondary action by our vital force. (*Organon of Medicine*, paragraph 65)[3]

In paragraph 63 of the *Organon of Medicine*[3], Hahnemann suggests a physiological explanation for the principle of therapeutic similitude (primary action of the drug followed by secondary and opposite action of the body), justifying its universal mechanism of action of drugs (biphasic action of drugs) on the automatic manifestation of "our life-preserving power" or "homeostasis" according to modern physiology:

> Every agent that acts upon the vitality, every medicine, deranges more or less the vital force, and causes a certain alteration in the health of the individual for a longer or a shorter period. This is termed *primary action*. […]. To its action our vital force endeavors to oppose its own energy. This resistant action is a property, is indeed an automatic action of our life-preserving power, which goes by the name of *secondary action* or *counteraction*. (*Organon of Medicine*, paragraph 63)[3]

Associating his empirical observations with hundreds of reports of involuntary homeopathic cures described in the literature and employing the inductive Aristotelian reasoning (*modus ponens*), Hahnemann enunciates the "principle of cure by similars" (*Organon of Medicine*, paragraphs 24-7)[3]: any medicine that cause, in their primary action, certain signs and symptoms in healthy individuals can be used, in their secondary action, to cure similar signs and symptoms in sick individuals (*similia similibus curentur*).

Therefore, the homeopathic method of treatment employs the secondary action or vital reaction of the body for therapeutic

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purposes by administering to ill individuals drugs that cause similar symptoms in healthy individuals in order to awaken a healing reaction of the body against their own disturbances or diseases.

By emphasizing that such secondary action of the body (vital reaction) is observed "in each and every instance with no exceptions" with ponderable or infinitesimal doses in both healthy and ill individuals, Hahnemann raised the principle of therapeutic similitude to the level of a "natural law of cure" (*Organon of Medicine*, paragraphs 58, 61, 110-2)[3].

In contrast, demonstrating the occurrence of evident worsening of disturbances or diseases after ceasing the palliative effect of drugs with contrary action to symptomatic manifestations ("principle of cure by contraries") (*Organon*, paragraphs 57-61)[3], Hahnemann reinforces the validity of homeopathic treatment according to the deductive Aristotelian reasoning (*modus tollens*) or "mode that affirms by denial":

> Important symptoms of persistent diseases have never yet been treated with such palliative, antagonistic remedies, without the opposite state, a relapse - indeed, a palpable aggravation of the malady - occurring a few hours afterwards. For a persistent tendency to sleepiness during the day the physician prescribed coffee, whose primary action is to enliven; and when it had exhausted its action the day - somnolence increased; - for frequent waking at night he gave in the evening, without heeding the other symptoms of the disease, opium, which by virtue of its primary action produced the same night (stupefied, dull) sleep, but the subsequent nights were still more sleepless than before; […] - weakness of the bladder, with consequent retention of urine, was sought to be conquered by the antipathic work of cantharides to stimulate the urinary passages whereby evacuation of the urine was certainly at first effected but thereafter the bladder becomes less capable of stimulation and less able to contract, and paralysis of the bladder is imminent; - with large doses of purgative drugs and laxative salts, which excite the bowels to frequent evacuation, it was sought to remove a chronic tendency to constipation, but in the secondary action the bowels became still more confined; [...] How often, in one word, the disease is aggravated, or something even worse is effected by the secondary action of such antagonistic (antipathic) remedies, the old school with its false theories does not perceive, but experience teaches it in a terrible manner. (*Organon of Medicine*, paragraph 59)[3]

According to the modern pharmacology, the "primary action" described by Hahnemann corresponds to the "therapeutic, adverse, and side effects" of conventional drugs, while the "secondary action" corresponds to the "rebound effect" or "paradoxical reaction" of the body, observed after discontinuation of several classes of drugs that act contrary (palliative or antipathic) to the signs and symptoms of diseases.

By definition, the term "rebound" is defined as the reversal of response upon withdrawal of a stimulus, while "rebound effect" means the production of increased negative symptoms when the drug ends or the patient no longer responds to it: if a drug produces a rebound effect, the condition it was used to treat may come back even stronger when the palliative drug is withdrawn (discontinued) or loses effectiveness (development of tolerance or tachyphylaxis).

The rebound effect manifests itself at different intervals (hours to weeks) after the end of the biological effect of the drug (pharmacological half-life), and its duration also varies. The rebound effect presents an intensity or frequency a few times higher than the corresponding baseline symptoms suppressed by the primary action of the antipathic drug. Although the rebound effect only occurs in a minority of susceptible individuals, it becomes an epidemiological concern when considering the exceedingly broad use of palliative treatments by the population.

Following the deductive Aristotelian reasoning employed by Hahnemann to validate the homeopathic healing principle, since 1998, we have been scientifically substantiating the "similitude in modern pharmacology" through the systematic study of the rebound effect (paradoxical reaction) of many classes of drugs[4-14], confirming the homeopathic postulate and the principle of similitude: a universal occurrence of strong secondary and opposite action of the body after ceasing the primary action of the drugs (Figure 1).

Illustrating the rebound phenomenon[4-14], it is observed that drugs classically used for the treatment of *angina pectoris* (e.g., β-blockers, calcium channel blockers, and nitrates) with beneficial effects through their primary action might trigger a paradoxical increase of the frequency and intensity of chest pain after its discontinuation. Drugs used for *arterial hypertension* (e.g., α-2 agonists, β-blockers, ACE inhibitors, MAO inhibitors, nitrates, sodium nitroprusside, and hydralazine) might produce rebound arterial hypertension after the end of the primary biological effect (half-life). *Antiarrhythmic* drugs (e.g., adenosine, amiodarone, β-blockers, calcium channel blockers, disopyramide, flecainide, lidocaine, mexiletine, moricizine, and procainamide) may trigger rebound exacerbation of basal ventricular arrhythmias. *Antithrombotic* drugs (e.g., argatroban,

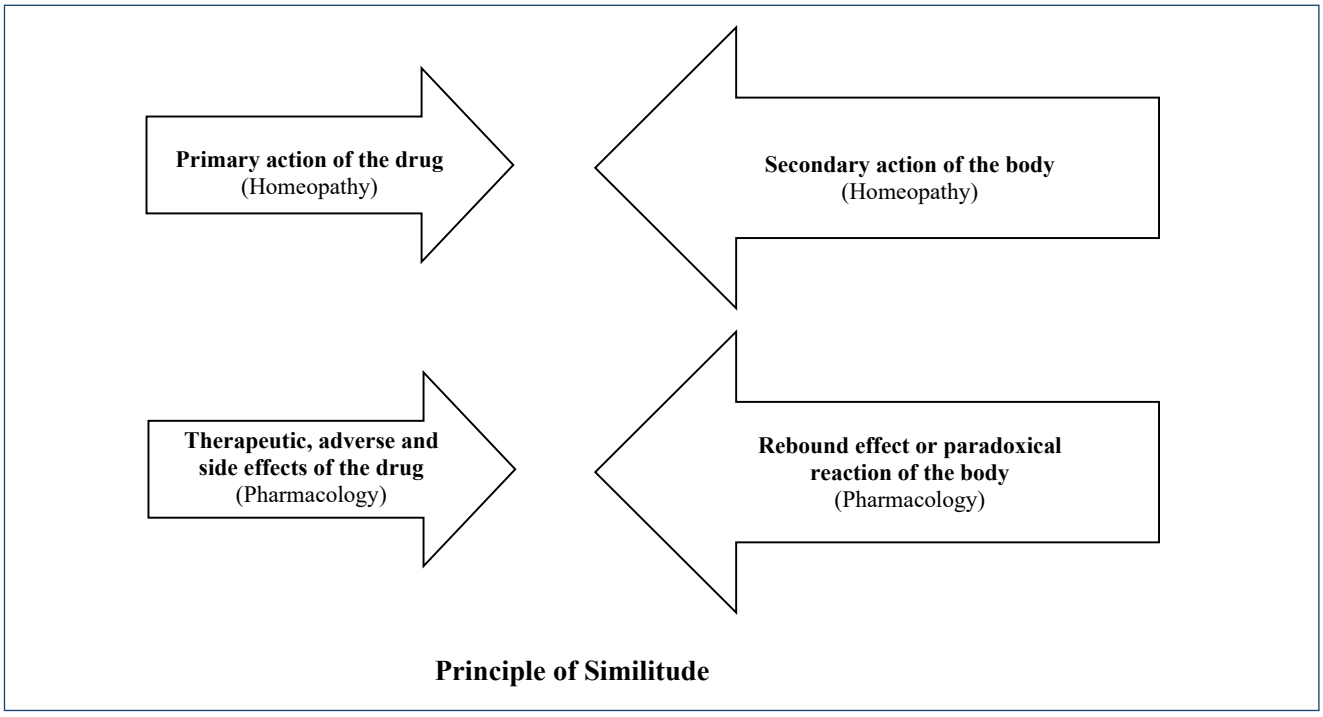

Figure 1. Universal mechanism of action of drugs (biphasic action of drugs): primary action of the drug followed by secondary and opposite action of the body (principle of similitude).

bezafibrate, heparin, salicylates, warfarin, and clopidogrel) might promote thrombotic complications as a result of the rebound effect. Drugs with primary *pleiotropic* or *vasoprotective* action (statins) might cause rebound endothelial dysfunction, resulting in predisposition to paradoxical vascular accidents.

Analogously, discontinuation of *anxiolytics* (e.g., barbiturates, benzodiazepines, and carbamates), *sedative-hypnotics* (e.g., barbiturates, benzodiazepines, morphine, promethazine, and zopiclone), *stimulants of the central nervous system* (e.g., amphetamines, caffeine, cocaine, mazindol, and methylphenidate), *antidepressants* (e.g., tricyclic, MAO inhibitors, and selective serotonin reuptake inhibitors), or *antipsychotics* (e.g., clozapine, phenothiazines, haloperidol, and pimozide) might cause rebound aggravation of the original condition after the end of their primary therapeutic action.

Anti-inflammatory agents (e.g., steroids, ibuprofen, indomethacin, paracetamol, and salicylates) might trigger a paradoxical increase of inflammation and rebound thrombosis (e.g., ibuprofen, indomethacin, diclofenac, salicylates, rofecoxib, and celecoxib) as a function of their primary platelet anti-aggregation effect.

Analgesics (e.g., caffeine, calcium channels blockers, clonidine, ergotamine, methysergide, opiates, and salicylates) might trigger rebound hyperalgesia. *Diuretics* (e.g., furosemide, torasemide, and triamterene) might cause sodium and potassium rebound retention, with consequent increase in the plasma volume and the blood pressure. *Bronchodilators* (e.g., short- and long-acting β-adrenergic agonists, sodium cromoglycate, epinephrine, ipratropium, and nedocromil) might promote rebound bronchoconstriction as a paradoxical reaction to discontinuation.

Anti-dyspeptic (e.g., antacids, H2 antagonists, misoprostol, sucralfate, and proton-pump inhibitors) might trigger rebound increase of hydrochloric acid production and gastrin, with worsening of the original condition. *Antiresorptive* drugs used for the treatment of osteoporosis (e.g., bisphosphonates, denosumab, and odanacatib) might cause paradoxical atypical fractures due to the rebound increase of osteoclast activity.

Discontinuation of drugs for the treatment of *multiple sclerosis* (e.g., glucocorticoids, interferon, glatiramer acetate, natalizumab, and fingolimod) might cause rebound increase of the inflammation with exacerbation of clinical symptoms and increase of demyelination lesions. *Immunomodulatory* agents (e.g., recombinant monoclonal antibodies and tumor necrosis factor inhibitors) indicated for the treatment of psoriasis might trigger rebound psoriasis after discontinuation. The list of examples is much longer[4-14].

According to these examples, the rebound effect, a universal and automatic physiological mechanism to maintain a constant

internal environment or homeostasis, is liable to be elicited by all types of palliative drugs. Due to the high intensity of primary action of drug, such paradoxical reaction might induce severe and eventually fatal adverse events.

Exemplifying these iatrogenic events, the long-acting β-adrenergic bronchodilators cause 1 episode of fatal rebound bronchospasm per 1,000 patient-years of use, corresponding to 4,000–5,000 deaths in 2004 in the United States alone, and 40,000–50,000 deaths worldwide[5,7]. Selective serotonin reuptake inhibitor antidepressants cause 5 rebound suicidal manifestations per 1,000 patient-years of use among adolescents, corresponding to 16,500 cases of suicidal ideation or behavior in 2007 in the United States alone[5,8]. Bisphosphonates cause 1–3 episodes of paradoxical atypical fractures per 1,000 patient-years of use[11].

Assuming that the principle of therapeutic similitude is a "natural law of cure," since 2003, we have been suggesting the homeopathic use of modern drugs employing the rebound effect in a therapeutic way[15-19], proposing to administer, in ultra-diluted doses, drugs that present, in their primary action, a set of signs and symptoms similar to the manifestations of sick individuals.

The proposal entitled "New Homeopathic Medicines: Use of modern drugs according to the principle of similitude" encompasses 1,250 modern drugs and has been available, since 2021, in three free-access digital books indexed in the Virtual Health Library (i.e., PAHO, WHO, and BIREME)[20]: "Scientific basis of the principle of similitude in modern pharmacology,"[21] "Homeopathic materia medica of modern drugs,"[22] and "Homeopathic repertory of modern drugs."[23]

To test the clinical and scientific validity of this proposal, we developed a randomized controlled trial employing potentized estrogen (17-β estradiol) for the treatment of endometriosis-associated pelvic pain[24], in view of the fact that estrogen causes as an adverse event a set of signs and symptoms similar to the endometriosis syndrome, observing significant improvement compared with placebo in relation to pain, depression, and quality of life[25].

We hope that these studies will be useful to all homeopathic doctors and researchers in the scientific validation of the therapeutic similitude and enable new applications for the homeopathic treatment.

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SHORT COMMUNICATION

https://doi.org/10.1590/1806-9282.20211292

Public interest in "early treatments" for coronavirus disease 2019 in Brazil: insights from Google Trends

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INTRODUCTION

The first case of coronavirus disease 2019 (COVID-19) in Brazil was reported on February 26, 2020, in the city of São Paulo, and the outbreak spread rapidly to other cities. At the time of this writing (November 4, 2021), 21,835,785 reported cases and 608,235 associated deaths had been confirmed in the country. The COVID-19 outbreak in Brazil has been characterized by sensationalism, fear, and misinformation[1]. Social media has been extensively used to support the false usefulness of the "early treatments" intended to prevent COVID-19 and cast doubt on the methods capable of mitigating the pandemic, such as the use of masks and social distancing[2]. These "early treatments" include the use of drugs with no proven efficacy for COVID-19, such as ivermectin, chloroquine, hydroxychloroquine (HCQ), and azithromycin. Ivermectin is a widely used drug for the treatment and control of many parasitic diseases[3]. Chloroquine is used to prevent and treat malaria and has efficacy as an anti-inflammatory agent for treating some inflammatory diseases such as rheumatoid arthritis and lupus erythematosus. Azithromycin is a broad-spectrum macrolide antibiotic used widely in the treatment and prevention of certain bacterial infections[4]. Although HCQ, chloroquine, and azithromycin have been used to treat patients with COVID-19 during the early pandemic period[4], recent systematic review and meta-analysis studies have not been able to support evidence for the efficacy of these drugs in the treatment and prevention of COVID-19[3,5-7]. Moreover, the irrational use of ineffective "early treatments" to prevent the disease can lead to bacterial resistance and adverse reactions[8].

In the present communication, we analyzed the public interest in ivermectin, chloroquine, HCQ, and azithromycin as early treatments during the COVID-19 pandemic. These drugs have been proposed as possible therapies for COVID-19, and even without proven efficacy for the treatment and prevention of the disease, they have been cited among the most commonly known drugs among the Brazilian population[9].

METHODS

Public interest was measured using Google Trends, a popular tool that provides information on frequencies of Internet queries from users on the Google search engine[10]. Data on COVID-19 were obtained from Cota[11], based on official sources from the Brazilian Ministry of Health. Statistical analysis of data was carried out using R version 4.1.1.

We performed a Google Trends search on November 8, 2021, to observe trends in the Internet searches in Brazil for Portuguese language versions of the terms "ivermectin," "chloroquine," "hydroxychloroquine," and "azithromycin" from January 6, 2019 until November 6, 2021. This period corresponds to the epidemiological week (EW) 2–52 in 2019, 1–53 in 2020, and 1–44 in 2021 (by convention, an EW is counted from Sunday to Saturday). Google Trends measures the public interest of a particular search query in relative search volume (RSV). RSV of a search term in a given week is measured on a scale of 0–100 based on its popularity compared to its peak search volume over a specified period. For example, the RSV for the search query "ivermectin" is 100 in the EW 28 of 2020 and 62 in the EW 29 of 2020. This means that in the EW 29 of 2020, this search term was 62% as popular as it was in the most popular week (EW 28 of 2020).

Generalized additive models (GAMs) were used to assess the association between the RSV for each drug and the weekly reported cases of COVID-19 infection. In this case, correlation coefficients are inappropriate due to the nonlinear relation between these series, and GAM provides more flexibility

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in the shape of the relationships between the variables compared to traditional models[12]. The mathematical formulation of the GAM model is given by $y_t=a+s(\log x_t)+e_t$. The variable y_t corresponds to the number of COVID-19 cases reported at the week t, x_t is the RSV for a given drug at the week t, a is a constant parameter, s is a smooth function, and e_t are independent and identically distributed error terms. The variable x_t was log-transformed to avoid the effect of outliers. In our analysis, GAMs were fitted using the "mgcv" package of R software. The model adequacy was assessed by plotting residuals versus fitted values and QQ plots, and the model fit was evaluated by the proportion of the null deviance explained (D).

As secondary data available online were used, the research was not submitted to an Ethics Committee on Human Research.

RESULTS

Figure 1 describes the weekly RSV for these four drugs. The dashed vertical line in the figure indicates the week when the World Health Organization (WHO) declared COVID-19 a global pandemic (March 11, 2020). We can note that the RSV for "ivermectin," "chloroquine," and "hydroxychloroquine" are close to zero before the dashed line, but weekly Google searches for "chloroquine" and "hydroxychloroquine" increased substantially just after that point. The peak of searches of the term "ivermectin" was reached when the public interest in chloroquine and HCQ decreased. Public interest in azithromycin was already substantial before the pandemic period, but it is observed that the number of searches for this term also increased after the WHO declaration.

Figure 2 compares the weekly RSV for the four drugs from February 23, 2020, until November 6, 2021 (EWs 9–53 in 2020 and EWs 1–44 in 2021, upper panel) and the time series of the weekly reported cases of COVID-19 in Brazil (lower panel). In Figure 2, we can note that the highest search volumes for ivermectin, HCQ, and azithromycin in the year 2021 occurred around EWs 11 and 12 (in mid-March). These peaks coincide with a period with a large number of notifications of COVID-19 (Figure 2B).

Figure 3 shows smoothed plots for the weekly Google searches for each drug versus the number of reported cases of COVID-19 infection in Brazil. All graphs in Figure 3 show a significant association between these variables so that the weeks in which there were more Internet searches for information on these drugs tended to be those with the highest number of COVID-19 notifications. In this analysis, we disregard the pandemic's exponential phase so that the time series used in the model started at EW 30 of 2020 (see Figure 2). Thus, in the model formulation, we consider t=1, …, 68. We highlight that these models are used only to describe the shape of the association between these variables without establishing cause-and-effect relationships.

DISCUSSION AND LIMITATIONS

Although ivermectin, HCQ, chloroquine, and azithromycin have no proven beneficial effect for treating COVID-19[3,5-7]

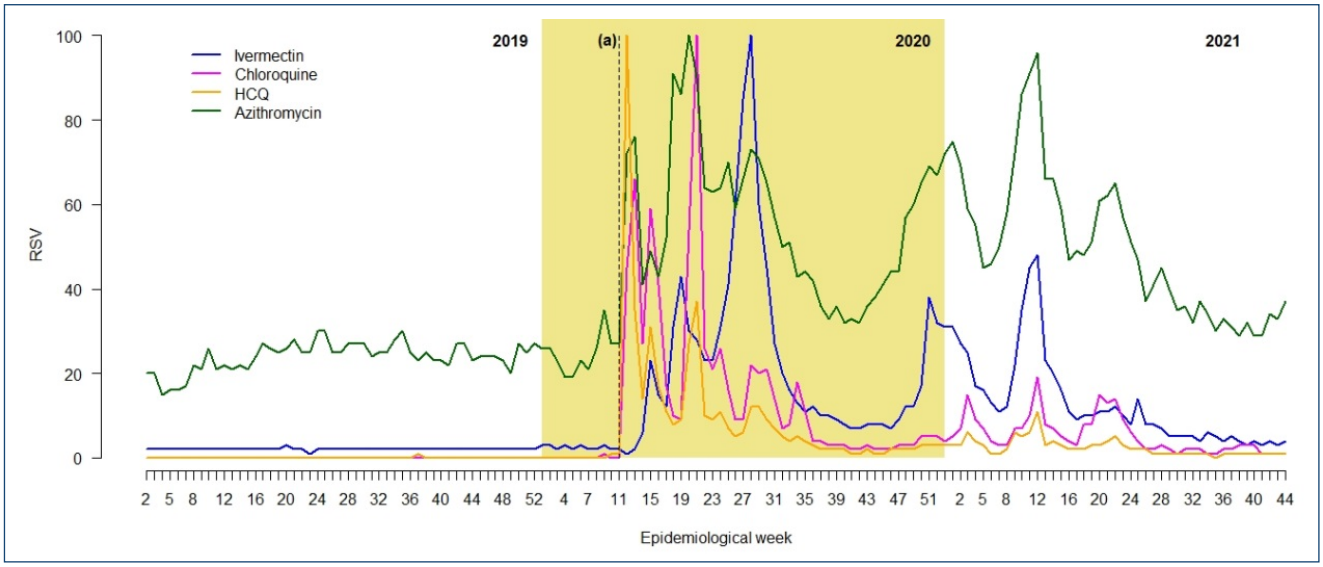

Figure 1. Weekly Google searches in Brazil for "ivermectin," "chloroquine," "hydroxychloroquine", and "azithromycin." The dashed vertical line labeled with an (a) indicates the week when the World Health Organization declared coronavirus disease 2019 a global pandemic (March 11, 2020).

their use as an "early treatment" for the disease has unfortunately been encouraged in Brazil by many politicians and religious leaders[13,14]. For example, Manaus, the capital of the state of Amazonas, bought US$71,000 in ivermectin for the treatment of COVID-19 and did not require a bidding process to reduce costs[13]. In April 2021, the Brazilian Senate created a Parliamentary Commission of Enquiry (CPI-Pandemia, acronym in Portuguese) to investigate actions and omissions of the federal government in tackling the pandemic, the collapse of healthcare in the state of Amazonas earlier this year, and irregularities in the use of public resources by states and municipalities. At the request of Senator Omar Aziz, President of the CPI-Pandemia, the Brazilian Federal Pharmacy Council produced a report on the sale of medicines associated with the treatment of COVID-19[15]. This report showed an 857% increase in ivermectin sales in the 12 months following the first recorded case of the disease in Brazil (March 2020). In this same period, the report showed a 126% increase in HCQ sales and a 71% increase in azithromycin sales. The report did not provide sales data for chloroquine, as this drug is distributed by the public health system and is not marketed to consumers by the private health sector. Despite this, the findings presented by the Brazilian Federal Pharmacy Council are consistent with those shown in Figure 1, which show that the public interest in "early treatments" for COVID-19 through Google search activity grew dramatically as soon as the WHO declared COVID-19 a global pandemic.

The increasing interest in ivermectin in the early months of the pandemic was largely motivated by the online publication of an article about its *in vitro* effect against the severe acute respiratory syndrome – associated coronavirus on April 3, 2020 (EW 14, 2020)[16] and by the subsequent media dissemination of the results of this research, which presented this drug as a promising treatment for the disease despite the lack of high-quality evidence. After the exponential phase of the pandemic (EWs 9–29, 2020), the public interest in "early treatment" is most intense in the periods with the highest notifications of COVID-19 cases (Figure 3).

The differences between officially reported cases of COVID-19 and the number of actual infections in the Brazilian population can be substantial, which could be viewed as a potential limitation of this study. This occurs due to many factors, including the underreporting of asymptomatic and mild cases, especially those that do not present for medical care, the insufficient

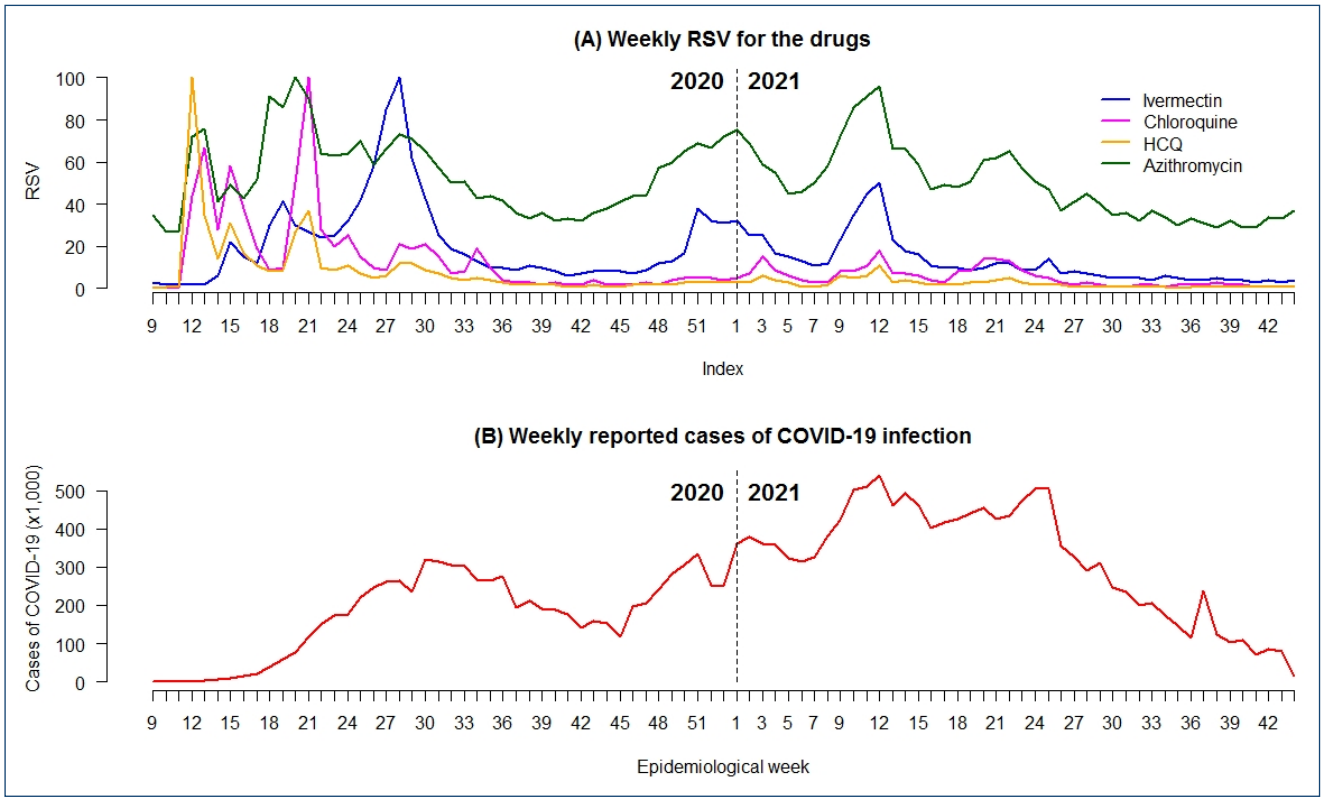

Figure 2. Panel (A) shows the weekly Google searches in Brazil for "ivermectin," "chloroquine," "hydroxychloroquine", and "azithromycin" from February 23, 2020 until November 6, 2021. Searches are presented as relative search volumes from 0 (least) to 100 (the highest number of searches). Panel (B) shows the weekly reported cases of coronavirus disease 2019 infection in Brazil in the same period of time.

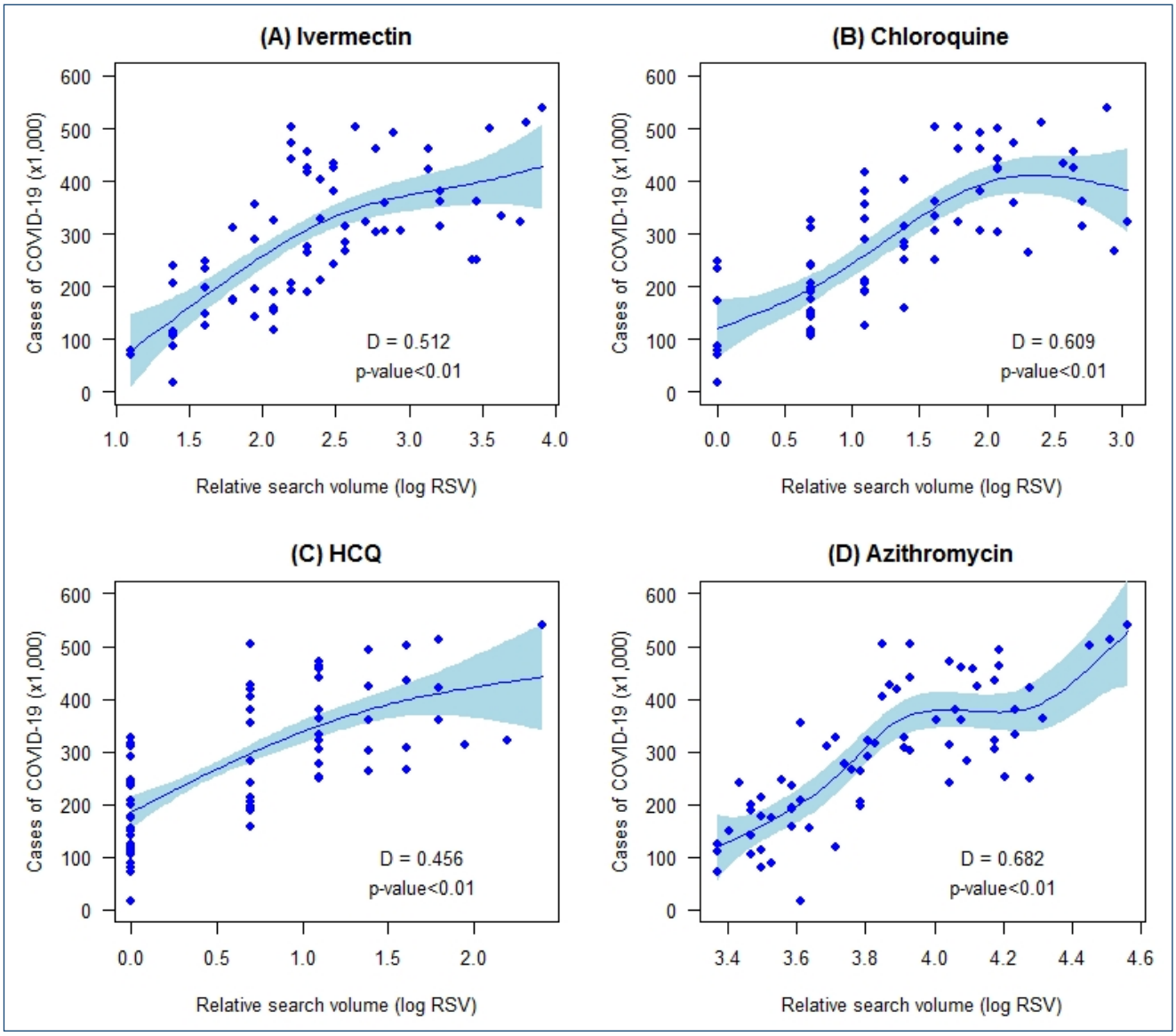

Figure 3. Generalized additive model analysis of the weekly reported cases of coronavirus disease 2019 infection in Brazil as a function of the weekly Google searches (in log scale) for (A) "ivermectin," (B) "chloroquine," (C) "hydroxychloroquine", and (D) "azithromycin" from July 19, 2020 until November 6, 2021. The shaded areas are error bands, panel (D) denotes the proportion of the null deviance explained by each model, and the p-values correspond to the approximate significance of smooth terms.

number of screening tests, false negative laboratory test results, and delays in reporting cases, among others.

CONCLUSIONS

Despite the limitations discussed previously, our findings suggest that Google Trends may be a useful tool for the continuous surveillance of the population's interest in inappropriate treatments for COVID-19 and, in an indirect manner, the consequent off-label use of medicines for this disease. The insights into the population's behavior provided by Google Trends can help create healthcare policies and information sources.

AUTHORS' CONTRIBUTIONS

EZM: Conceptualization, Formal Analysis, Writing – original draft, Writing – review & editing. **DCA**: Writing – original draft, Writing – review & editing. **MLZ**: Writing – original draft, Writing – review & editing.

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ORIGINAL ARTICLE

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Prognostic assessment of tumor markers in lung carcinomas

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SUMMARY

BACKGROUND: Serum tumor markers are molecules that are secreted by tumor cells and may be present in small amounts in the serum of healthy individuals. Their role as prognostic factors in lung cancer remains controversial.

OBJECTIVE: To assess the prognostic role of CEA, CA 19-9, CA 15-3, and CA 125 in non-squamous non-small cell lung cancer.

PATIENTS AND METHODS: A total of 112 patients with non-squamous non-small cell lung cancer from two Oncology Centers were retrospectively analyzed. Tumor marker levels were measured prior to treatment. Data regarding clinical characteristics and overall survival were collected.

RESULTS: Median overall survival of all patients was 15.97 months. Pre-treatment elevations of CA 125 and CA 15-3 were associated with shorter overall survival (p=0.004 and p=0.014, respectively). Single CEA and CA 19-9 elevations were not associated with a worse prognosis. Patients with two or more elevated markers had a statistically significant decrease in overall survival (p=0.008). In the multivariate analysis, smoking status and number of positive tumor markers at diagnosis were independently associated with a worse prognosis.

CONCLUSION: High pre-treatment levels of tumor markers were correlated with decreased survival in patients with non-squamous non-small cell lung cancer.

KEYWORDS: Lung neoplasms. Carcinoembryonic antigen. CA-19-9 antigen. Mucin-1.

INTRODUCTION

Lung cancer is the most lethal neoplasm worldwide[1]. Despite recent therapeutic advancements, the life expectancy of these patients remains short, since approximately 80% of cases are diagnosed at an advanced stage. Clinical and/or pathological staging is considered the main prognostic factor. Other important factors are performance status, weight loss, smoking status, and some histopathological and molecular traits[2-4].

Tumor markers are molecules, usually peptides, which are secreted by tumor cells. Their role as a prognostic or therapeutic response monitoring tool in other neoplasms is already widely known. In lung cancer, their use is not routinely recommended by oncology societies[5,6], and hence, in general, they are scarcely employed.

In view of the differing conclusions published on the prognostic value of tumor markers in lung cancer, conducting a new study on the subject could contribute to better understanding the topic.

This study aims to assess the importance of CEA, CA 19-9, CA 15-3, and CA 125 markers as prognostic factors in patients with non-squamous non-small cell lung cancer (NSCLC).

METHODS

This is a retrospective cohort study based on the data collected from medical records in two different institutions. The population comprised 112 patients with non-squamous NSCLC, diagnosed between May 2002 and July 2019. Samples containing the markers were collected from the patients before starting the treatment for cancer.

The following data were collected: name, age, sex, duration of survival, clinical or pathological stage of the neoplasm, histological type, smoking status, and the number of positive markers at diagnosis.

Patients from *Clínica São Germano* had their samples collected at different laboratories located in the State of São Paulo. The following marker level values were considered "positive": CEA>5 ng/mL, CA 19-9>37 UI/mL, CA 125>35 UI/mL, and CA 15-3>30 UI/mL. For patients from *Santa Casa de Misericórdia*, the samples were collected by the institution laboratory, and the following marker level values were considered "positive": CEA>5 ng/mL, CA 19-9>37 UI/mL, CA 125>35 UI/mL, and CA 15-3>25 UI/mL.

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Inclusion criteria

- Histologically documented diagnosis of non-squamous NSCLC;
- Age³18 years;
- Having had at least one of the four markers dosed before starting treatment;
- Patients diagnosed with another localized malignant neoplasm prior to lung cancer were allowed, as long as they had received treatment and showed no evidence of relapse for at least 5 years.

Exclusion criteria

- Incomplete data in medical records;
- Diagnosis of another metastatic malignant neoplasm (synchronous or prior to the lung cancer diagnosis);
- Refusal to sign the VICF.

Statistical analysis

The duration of overall survival (OS) was calculated as the time from the date of diagnosis until the date of death from any cause. Data were collected until May 2020.

Variables were analyzed descriptively. For quantitative variables, this analysis was performed by observing the minimum and maximum values and calculating means, standard deviations, and medians. For qualitative variables, absolute and relative frequencies were calculated.

To test the homogeneity among proportions, the chi-square test or Fisher's exact test was used. To study the association of markers with survival time, the Kaplan–Meier survival curve and the univariate Cox regression model were used. The multivariate study was performed by using the multivariate Cox model. Variables with $p<0.10$ in the univariate analysis were selected. A "stepwise" selection process was applied in order to produce the final model. The significance level used for the tests was 5%. The software program used in the analyses was SPSS 17.0.

RESULTS

Altogether, 112 patients aged between 28 and 91 years (mean of 66.19 years with a standard deviation of 12.02 years and a median of 66.50 years) were evaluated. Other clinical characteristics of the population are described in Table 1.

CEA was measured in 109 patients. Of these, 64.2% showed an increased level of the marker. The CA 19-9 level, in turn, was measured in 97 patients and found to be high in 30.9% of them. The CA 125 marker level was measured in 97 individuals and found to be increased in 56.7% of them. The CA 15-3 level was measured in 91 cases and tested positive in 50.6% of patients (Table 1).

Overall survival ranged from 25 days to 137 months (mean of 27.08 months with a standard deviation of 28.66 months and a median of 15.97 months). At the time of analysis, 83 patients (74.1%) had died.

Table 2 shows the "Hazard Ratio" values for survival according to the univariate Cox model. Smoking status, staging, high CA 125 levels, high CA 15-3 levels, and two or more of the four increased markers were the factors associated with worse survival. The median OS of patients with a negative CA 125 and a positive CA 125 were 18.84 months and 11.93 months ($p=0.004$), respectively. In patients with normal CA 15-3 level, the median OS was 18.57 months; however, in patients with a higher marker level ($p=0.014$), the median OS was 13.44

Table 1. Patients' clinical traits.

	%
Sex	
Male	50.0
Female	50.0
Histological subtype	
Adenocarcinoma	99.1
Large cell carcinoma	0.9
Staging	
I	5.4
II	6.3
IIIA	8.9
IIIB	10.7
iv	68.8
Smoking status	
No	38.9
Yes	61.1
Marker positivity frequency	
CEA	64.2
CA19-9	30.9
CA125	56.7
CA15-3	50.6
Number of positive markers	
None	19.1
One	21.4
Two	20.2
Three	23.8
Four	15.5

months. The increase in CEA and CA 19-9 levels alone did not correlate with a worse prognosis (p=0.072 and p=0.154, respectively). All four marker levels were collected in 84 patients. The median OS in patients with no positive marker was 28.53 months. The median OS in patients with one positive marker was 19.55 months, and it was 12.80 months in the group with two or more positive markers (p=0.008).

The variables such as smoking status, staging, CEA level, CA 125 level, CA 15-3 level, and the number of positive markers were used in the multivariate Cox model. The variables selected by the stepwise method were smoking status and the number of positive markers (Table 3).

The risk of death was twice as high among smokers (p=0.017). Patients with two or more high marker levels also had an approximately twofold increase in the risk of death when compared to patients with no positive marker (p=0.024).

DISCUSSION

In this work, we assessed the tumor markers CEA, CA 19-9, CA 125, and CA 15-3 in non-squamous NSCLC. The prognostic value of tumor markers in lung cancer is controversial. The most studied one is CEA, followed by CA 125. CA 15-3 and CA 19-9 have been very poorly assessed in this context.

We found that CA 125 and CA 15-3 were correlated with shorter OS in the univariate analysis.

CA 125 was positive in 56.7% of patients. Several publications report sensitivities between 31.7 and 55%[7-11]. In our patients, having high CA 125 levels before treatment was a negative prognostic factor. Other authors have obtained similar results in both early and advanced diseases. Of the four markers studied, CA 125 was the one with the most consistent results in the literature regarding its prognostic role[10,12].

Few authors have studied CA 15-3 in lung neoplasms. In our survey, CA 15-3 was positive in 50.6% of cases, which is consistent with the findings published by other authors[8,14]. Regarding survival, we found that having high pretreatment CA 15-3 levels led to a statistically significant decrease in the median OS. Our findings differ from the results published by Gross et al. who found no relationship between CA 15-3 levels and duration of survival[14].

CEA was positive in 64.2% of our cases. This result is in accordance with the results of several publications in which the CEA sensitivity ranged from 41 to 77%[7-11,14]. Nevertheless, having increased CEA levels was not considered as a negative prognostic factor. Several researchers have already assessed the prognostic utility of CEA in lung neoplasm, in both early and advanced diseases, albeit with conflicting results[10,11,13,15-23].

Table 2. "Hazard ratio" values for survival: univariate Cox model.

Variable	Hazard ratio	95%CI	p
Age			
	1.01	(0.99; 1.02)	0.532
Sex			
Female	1.00	–	–
Male	1.35	(0.87; 2.10)	0.177
Institution			
CSG	1.00	–	–
HSC	1.23	(0.76; 1.99)	0.407
Smoking status			
Non-smoker	1.00	–	–
Smoker	2.02	(1.23; 3.31)	0.006
Staging			
I–II	1.00	–	–
IIIA	7.12	(1.51; 33.53)	0.013
IIIB	10.37	(2.33; 48.93)	0.002
IV	10.15	(2.47; 41.66)	0.001
CEA			
Negative	1.00	–	–
Positive	1.54	(0.96; 2.48)	0.074
CA 19-9			
Negative	1.00	–	–
Positive	1.44	(0.87; 2.38)	0.156
CA 125			
Negative	1.00	–	–
Positive	2.04	(1.25; 3.35)	0.005
CA 15-3			
Negative	1.00	–	–
Positive	1.87	(1.13; 3.10)	0.016
Number of positive markers			
None	1.00	–	–
One	1.15	(0.45; 2.91)	0.770
Two or more	2.56	(1.20; 5.48)	0.016

Table 3. "Hazard ratio" values for survival: multivariate Cox model.

Variable	Hazard ratio	95%CI	p
Smoking status			
Non-smoker	1.00	–	–
Smoker	2.01	(1.13; 3.58)	0.017
Number of positive markers			
None	1.00	–	–
One	1.25	(0.49; 3.18)	0.637
Two or more	2.41	(1.12; 5.15)	0.024

CA 19-9 was the marker that increased the least frequently in our study (30.9%). In the few analyses of the role played by CA 19-9 in lung cancer published in the literature, the sensitivity ranged from 9.3 to 31%[8,12,13,24]. In our sample, we found no association between high CA 19-9 levels and prognosis. For Ma et al., who found a sensitivity of only 5% for CA 19-9 in patients with stage I NSCLC, an increase in the levels of this marker did not interfere with survival[15]. In contrast, other authors were able to correlate higher levels of this marker with a worse prognosis[12,24].

Finally, we found that, in patients who had at least two markers whose levels were high, survival was significantly lower in the univariate analysis. This remained to be an independent prognostic factor in the multivariate analysis. Other authors, when evaluating different combinations of markers, have also reported similar data[11,12,14,25].

Our findings, however, have limitations. This is a retrospective analysis that considers only two institutions. Another limiting factor was the use of different laboratories for the collection of samples containing the markers. Since we used qualitative data, this bias could be mitigated. Another relevant issue was the lack of data on performance status in our study. Performance status is known to be one of the main prognostic factors in oncology. In our case, the information about performance status at diagnosis was not available in a sufficient number of cases; therefore, we decided not to collect such data.

CONCLUSION

Having high levels of tumor markers prior to treatment was considered a poor prognostic factor in non-squamous NSCLC.

AUTHORS' CONTRIBUTIONS

FBS: Study design, conceptualization, data curation, formal analysis, investigation, methodology, project administration, writing – original draft, writing – review and editing. **RSJ:** Supervision, validation, writing – review and editing.

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ORIGINAL ARTICLE

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Tocilizumab is useful for coronavirus disease 2019 patients: the key point is timing

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SUMMARY

OBJECTIVE: In coronavirus disease 2019, a rapidly progressive inflammatory process is considered to be the main cause of organ damage and mortality. Therefore, the importance of anti-inflammatory treatments such as tocilizumab is increasing.

METHODS: A total of 107 patients who received tocilizumab between March 2020 and March 2021 were included in the study. The primary termination point was mortality. We compared surviving and deceased patients by the stage of the disease and where the drug was given (service or intensive care unit).

RESULTS: The mean age was 60.8±14.6 years (minimum 29 years, maximum 96 years). According to the WHO staging system, 16 (15%) patients had moderate, 47 (43.9%) patients had severe, 44 (41.1%) patients had a critical illness. Although all patients were admitted to the service, 26 (24.3%) patients received tocilizumab in the intensive care unit. Of 107 patients, 80 (74.7%) survived and 27 (25.2%) died. Mortality was found to be significantly higher in critical patients (96.3%), severe patients (3.7%), and moderate patients (0%) ($p<0.001$). Peripheral oxygen saturation measured at admission was found to be significantly lower in patients who died. The initial saturations ($p=0.008$) were found to have independent effects on mortality.

CONCLUSION: The results showed that tocilizumab is an effective treatment option for coronavirus disease 2019 disease and reduces mortality, but the key point is timing.

KEYWORDS: Tocilizumab. Early treatment. Mortality. Peripheral oxygen saturation monitoring.

INTRODUCTION

After cases of novel coronavirus [coronavirus disease 2019 (COVID-19)/severe acute respiratory syndrome-coronavirus-2 (SARS-CoV-2)] pneumonia were reported in Wuhan City, Hubei Province, China, in December 2019, the World Health Organization (WHO) declared the outbreak as a "global pandemic" on March 11, 2020. The WHO reported 163,212,429 cases and 3,386,825 deaths until May 19, 2021[1].

At present, many antiviral agents have been tried for COVID-19, but none of them are sufficient and effective alone. Chloroquine and hydroxychloroquine have also been mainly used to treat COVID-19 infection, as they have been examined in the treatment of the previously seen SARS and Middle East Respiratory Syndrome (MERS) epidemic[2]. The lopinavir/ritonavir combination was used in the treatment of SARS and was also evaluated in the treatment of COVID-19[3]. Wang et al. used the combination of lopinavir/ritonavir, arbidol, and Shufeng Jiedu capsule in the treatment of COVID-19 and stated that this combination has some clinical benefits[4]. Favipiravir and Remdesivir are other drugs that are also used in the treatment of COVID-19. Some studies have shown that these drugs may have some advantages[5,6].

The occurrence of cytokine storm during COVID-19 infection is a hyperacute condition that causes mortality and needs to be prevented quickly. The cytokine storm, defined in the previous studies involving COVID-19 patients, has been better understood daily[7].

Due to the cytokine storm, which occurs during COVID-19 infection, alternative treatments are needed, which gradually increases the importance of anti-inflammatory drugs. It turned out that anti-inflammatory drugs are as important as antivirals. For this purpose, interleukin (IL)-1 receptor antagonists, (IL)-6

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receptor antagonists, Janus kinase inhibitors, granulocyte-macrophage colony-stimulating factor, anti-tumor necrosis factor-α, and corticosteroids were used in the treatment of COVID-19[8].

Tocilizumab is the most studied anti-inflammatory agent for the treatment of COVID-19 infection. It has also been primarily considered in the treatment of severe COVID-19 pneumonia, which is thought to have high (IL)-6 levels and is accompanied by a cytokine storm. However, the results of tocilizumab treatment are controversial. While it was found to be beneficial in some studies, other studies reported that it is not effective[9,10].

In this study, we evaluated our patients who used tocilizumab. In this study, we investigated whether tocilizumab has an effect on mortality in COVID-19 infection and whether the time of administration of tocilizumab treatment has an effect on mortality.

METHODS

COVID-19 treatment decision was made following the guideline by The Turkish Ministry of Health. This guide was first published in May 2020 and updated over time. The last update was done on May 7, 2021[11]. According to this guide, treatment regimens changed (Table 1).

Study design

This study was primarily designed for patients who were followed in the pandemic service. Patients who were admitted to the pandemic service and received tocilizumab between March 2020 and March 2021 were included in the study. Patients who were admitted directly to the intensive care unit (ICU) on admission were excluded from the study. Patients under the age of 18 years were excluded from the study. Data were analyzed retrospectively.

Initial oxygen (O_2) saturations were recorded at admission. Peripheral O_2 saturation measured at admission was used to define the term "late admission." Daily blood tests were done. Patients were classified according to the WHO classification system[12].

The primary termination point was determined as mortality. We thought that timing was important for tocilizumab treatment. Therefore, we compared the stages of the disease in which patients had taken the drug.

Statistical analyses were performed with the SPSS-20.0 program (SPSS, Chicago, IL, USA). Numerical data were expressed as mean and standard deviation, whereas categorical data were expressed as percentages or proportions. The chi-square test was used to analyze categorical variables. Comparison between three groups for numeric variables was made using the one-way ANOVA test. Factors having an independent effect on mortality were evaluated by linear regression analysis. A p-value <0.05 was considered to be statistically significant.

Ethics committee approval was received from Bolu Izzet Baysal University.

Table 1. Turkey's ministry of health coronavirus disease 2019 guide antiviral treatment suggestions.

Treatments			
	First choice	Second choice	Third choice
First suggestion	Hydroxychloroquine+ Azithromycin+ Oseltamivir	Lopinavir/ Ritonavir	Favipiravir
Second suggestion	Hydroxychloroquine+ Azithromycin+ Oseltamivir	Lopinavir/ Ritonavir	
Third suggestion	Hydroxychloroquine Azithromycin	Favipiravir	
Final suggestion	Favipiravir		

RESULTS

A total of 107 patients were included in the study. The mean age was 60.8±14.6 years (minimum 29 years, maximum 96 years). According to the WHO staging system, 16 (15%) patients had moderate, 47 (43.9%) patients had severe, 44 (41.1%) patients had a critical illness.

Of 107 patients, 97 were PCR-positive and 10 were antibody-positive; 78.5% of the patients were males (84) and 21.5% were females (23). Despite all patients hospitalized to service on admission, 26 (24.3%) received tocilizumab in the ICU.

Of 107 patients, 80 (74.7%) survived and 27 (25.2%) died. A total of 15 (14%) patients from survivors were discharged with an oxygen concentrator. When compared to the patients who take the tocilizumab in the ICU COVID service, it was observed that the mortality rate was 8.6% in service and 76.9% in ICU patients (Figure 1). The difference was statistically significant (p<0.001).

While 76.5% of the patients recovered completely, 14.8% were discharged with oxygen and received tocilizumab in the service. In contrast, 11.5% of those recovered completely, and 11.5% were discharged with oxygen receiving tocilizumab in the ICU. While 8.6% of the patients who received tocilizumab in the service died, 76.9% who received it in the ICU died. The difference was statistically significant (p<0.001).

The relationship between the characteristics of the patients and mortality was also examined in the study. Mortality was significantly higher in critical (96.3%) patients than in severe (3.7%) and moderate (0%) patients (p<0.001). Mortality was significantly higher for males (88.9%) than females (11.1%) (p=0.042). Initial O_2 saturation was significantly lower in deceased patients (p<0.001).

Factors having an independent effect on mortality were evaluated by linear regression analysis (Table 2). Initial saturation, highest ferritin, and lowest lymphocyte values had independent effects on mortality.

DISCUSSION

A hyperacute inflammatory process is observed in the course of COVID-19 disease. This process has been named cytokine release syndrome and hyperinflammatory syndrome in the previous studies[9,13,14]. These conditions have been previously seen in various diseases such as some viral diseases, sepsis, rheumatological diseases, and after the use of some drugs. In addition, lymphopenia, high CRP, ferritin, triglyceride, lactate dehydrogenase (LDH), and D-dimer values seen in COVID-19 can also be seen in these syndromes[14,15]. As a result, uncontrolled and massive release of inflammatory cytokines is seen, and this abnormal rise can be measured[16].

An abnormal immune response occurs in COVID-19 infection. Hyperactivation of T cells and uncontrolled and unpreventable cytokine release are held responsible for the poor prognosis of diseases[17]. Studies on viral load in SARS patients have shown that the prognosis worsens even as the

Table 2. Factors having an independent effect on mortality.

	B	t	p
Age	0.010	1425	0.157
Hospitalization day	-0.009	-0.601	0.549
Initial O_2 saturation	-0.051	-2716	0.008
Highest CRP	0.002	0.938	0.351
Highest ferritin	0.001	2733	0.007
Lowest lymphocyte	-1110	-3856	0.000

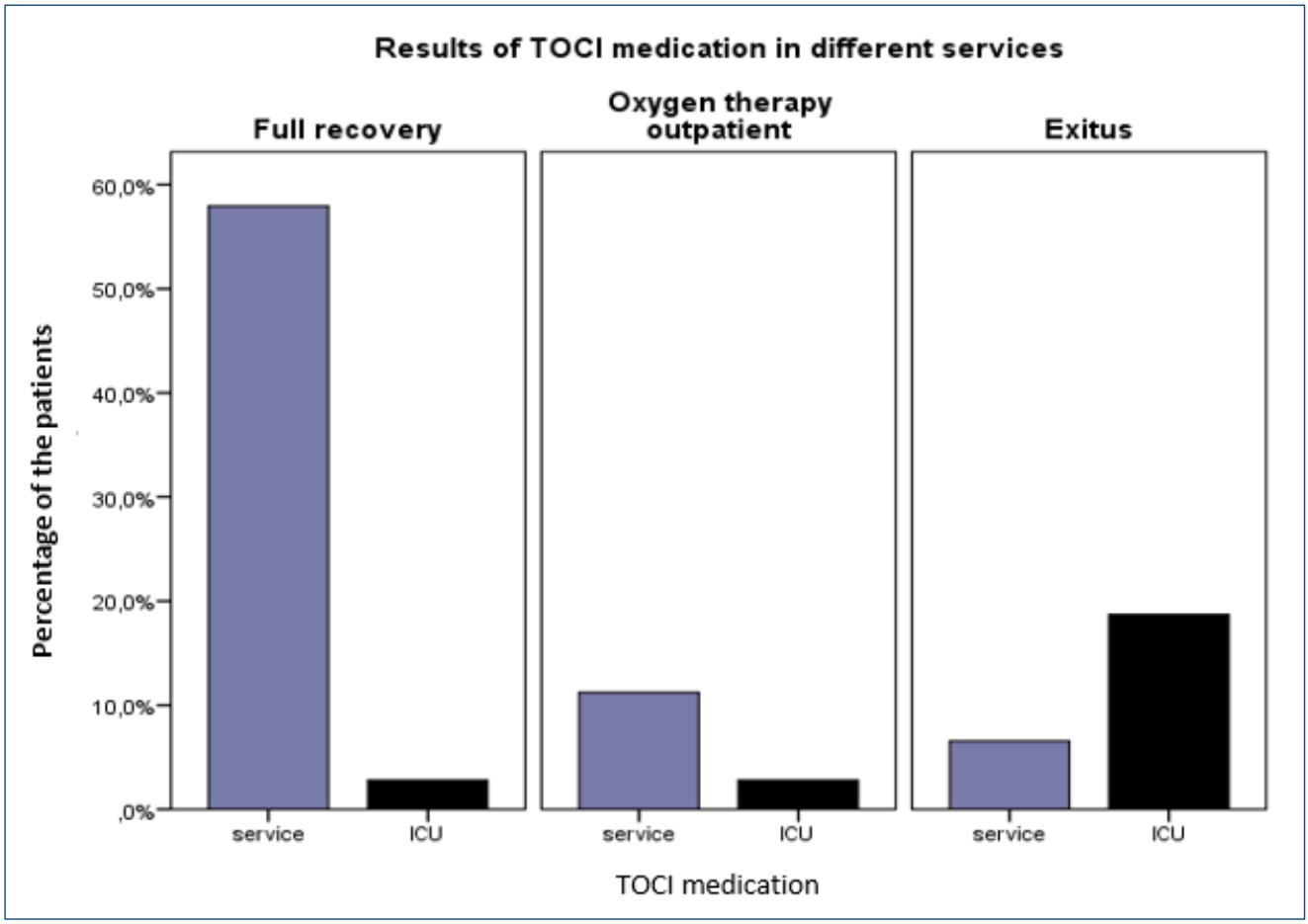

Figure 1. The comparison between ICU and service patients according to mortality, discharge from hospital with oxygen, and full recovery.

viral load of patients decreases due to high inflammation. These results suggest that anti-inflammatory therapy is more critical than antivirals[14].

Coronavirus infections had high expression of IL-6, including COVID-19, SARS-CoV, and MERS-CoV. IL-6 plays an important role in acute inflammation. It regulates acute inflammation. However, excessive IL-6 signaling leads to organ damage[14,15].

IL-6 inhibitor (tocilizumab) is recommended for anti-inflammation in COVID-19 patients. However, some of these studies recommend tocilizumab only for severely or critically ill patients[14,15]. Luo et al. used tocilizumab in 15 patients and stated that CRP values are decreased in all patients. Although seven of them were critically ill, they reported that only three patients died[7]. Xu et al. administered tocilizumab to 21 patients and reported that all patients survived[18]. In our study, the rate of COVID-19 cases with severe and critical diseases was over 40%, while 15%. All of these cases were treated with tocilizumab in the service or ICU. Unlike the studies above, anti-inflammatory treatment (tocilizumab) was applied to the moderate COVID-19 patients.

Klopfenstein et al. compared two groups, one taking standard therapy and the other traditional therapy with tocilizumab drug. They pointed out that in common therapy, group mortality and ICU admission are significantly higher[19]. Conversely, Colaneri et al. showed that tocilizumab did not affect ICU admission substantially or 7-day mortality[20]. Studies done in ICU patients have poorer outcomes. In their study conducted with 65 patients, Campochiaro et al. administered tocilizumab to 32 of them and compared the two groups. They found no significant difference when comparing mortality and clinical outcomes. However, all of the patients included in the study were patients who needed high-flow oxygen or noninvasive ventilation[21].

Gorgolas et al. similarly demonstrated that tocilizumab is beneficial in early administration[22]. In this study, when comparing the patients who took the tocilizumab in COVID service with those who took it in the ICU, it was observed that the mortality rate was 8.6% in service and 76.9% in ICU patients. The difference was statistically significant ($p<0.001$). It was noted that 26 of 27 patients who were died were critically ill. Only one of them was severe. Mortality was significantly higher in critical (96.3%) patients than in severe (3.7%) and moderate (0%) patients ($p<0.001$). We compared the deceased and survived patients according to initial O_2saturation. Initial O_2 saturation was significantly lower in deceased patients. Using linear regression analysis, it was observed that initial O_2 saturation, highest ferritin, and lowest lymphocyte values had independent effects on mortality.

Previous studies have shown that patients can benefit from tocilizumab even if they are treated in the ICU[23]. Although we recommend early treatment, we believe that every patient can receive tocilizumab. In our study, the fact that 76.9% of the patients given tocilizumab in the ICU passed away supports our argument that "the sooner, the better." It will be wiser to provide some patients with unnecessary treatment rather than expose them to mortal risk since we are not sure who will go worse.

For this reason, we suggest that the best approach is not to allow patients to progress, by starting anti-inflammatory therapy as soon as signs of inflammation appear. In studies related to this, tocilizumab was recommended for patients with CRP ≥75 mg/L and saturation <92%[24]. We suggest that even these values may be insufficient. We recommend tocilizumab for patients with a CRP greater than 50 mg/L and/or a peripheral O_2 saturation of less than 93. However, it should be kept in mind that some patients progress very rapidly. In this case, we suggest that the decision of the expert who follows up the patient is the most crucial criterion. We recommend that for patients with locally determined insufficient prognosis criteria, tocilizumab treatment can be started without waiting for these values to occur.

In addition, in our study, we showed that low peripheral O_2 saturation has an independent effect on mortality. We suggest that it would be beneficial to monitor the peripheral O_2 saturation at home by the patients themselves to prevent the late arrival of patients who are followed up or treated at home.

CONCLUSIONS

There is still no antiviral therapy that affects mortality. However, anti-inflammatory treatments affect mortality. It can be revealed that anti-inflammatory treatment is more important than antivirals; our study showed that early use of tocilizumab reduces mortality.

To reduce mortality, two simple steps are needed: (1) Monitoring peripheral O_2 saturation at home to avoid late hospitalization. (2) Tocilizumab treatment should be given before the cytokine storm occurs.

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AUTHORS' CONTRIBUTIONS

MG: Conceptualization, Data curation, Formal Analysis, Investigation, Methodology, Resources, Software, Supervision, Validation, Visualization, Writing – original

draft, Writing – review & editing. **UÖ:** Conceptualization, Data curation, Formal Analysis, Investigation, Methodology, Resources, Software, Supervision, Validation, Visualization. **ND:** Investigation, Methodology, Supervision, Writing – original draft, Writing – review & editing. **GC:** Investigation, Methodology, Writing – original draft, Writing – review & editing. **AC:** Conceptualization, Data curation, Writing – review & editing. **DK:** Conceptualization, Data curation. **KS:** Conceptualization, Data curation. **AK:** Conceptualization, Data curation, Investigation, Methodology, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. **CK:** Conceptualization, Investigation, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. **SG:** Conceptualization, Data curation.

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ORIGINAL ARTICLE

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Association between handgrip strength and body composition, physical fitness, and biomarkers in postmenopausal women with metabolic syndrome

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SUMMARY

OBJECTIVES: This study examined the association between handgrip strength and body composition, physical fitness, and biomarkers in postmenopausal women with metabolic syndrome.

METHODS: A total of 75 postmenopausal women were diagnosed with metabolic syndrome participated in this study. Muscle strength was assessed via a hydraulic grip strength dynamometer; physical fitness tests included a timed-up-and-go, arm curl test, and 30-s chair stand. Body composition was assessed via bioelectrical impedance, from which estimates of fat mass, body fat percentage, fat-free mass, and visceral fat area were determined. Fasting plasma glucose and glycated hemoglobin were measured via blood sample analyses. Multiple linear regression analyses were conducted using handgrip strength as the dependent variable and using body composition, physical fitness, and biomarkers as independent variables.

RESULTS: The results revealed that 52% of the total sample were classified as obese, 37.3% as overweight, and only 10.7% as normal weight. Significant correlations were present between handgrip strength and fat-free mass (p=0.002; R=0.590), mean blood pressure (p=0.002; R=0.450), and arm curl (p=0.001; R=0.795).

CONCLUSION: This study showed that handgrip strength was predictive of fat-free mass, blood pressure, and upper limb strength performance.

KEYWORDS: Metabolic syndrome. Blood pressure. Body weight. Muscle strength. Physical fitness.

INTRODUCTION

Metabolic syndrome is a physiopathology with high worldwide prevalence and is characterized by multifactorial and progressive risk factors[1]. Generally, metabolic syndrome is diagnosed when the individual has four of the following conditions: dyslipidemia, presenting high low-density lipoprotein (LDL) and triglycerides, and low high-density lipoprotein (HDL); overweight and obesity, especially with abdominal fat concentration; elevated blood pressure (BP); and insulin resistance, with high levels of fasting glucose[2]. Metabolic syndrome increases cardiovascular morbidity, with progressive development of atherosclerosis and coronary artery disease (CAD), hypertension, and increased risk of stroke[3].

Prospective studies have found that physical fitness is inversely related to the prevalence of metabolic syndrome[4]. This can be attributed to sedentary behavior, with high amounts of sitting and reduced physical activity, which may increase cardiometabolic risk[5]. Since cardiorespiratory fitness is generally used as an indicator of functional capacity, it is an independent predictive factor of improved cardiometabolic risk profile, associated with a higher HDL concentration, lower waist circumference, and fasting glucose[6]. Bentes et al.[7] reported in a previous study, with 40 postmenopausal women, that muscle strength was associated with the reduced serum glucose concentrations and might be a key indicator of metabolic health. Moreover, postmenopausal women exhibit an increase in visceral adipose tissue and proinflammatory cytokines increasing cardiovascular risk[8,9].

Since grip strength is generally used as an index of general muscle strength and frailty, it has been used in clinical trials and considered a simple approach to evaluate muscle function[7]. Studies that assessed grip strength found it to be

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associated with body composition parameters and physical fitness performance[7,10]. This is important to consider since previous studies reported associations between changes in body composition, especially the increases in body fat and metabolic syndrome[11]. Other studies revealed associations between handgrip strength and other chronic diseases, such as type 2 diabetes, hypertension, and depression[12,13]. Therefore, the purpose of this study was to examine the association between handgrip strength and body composition, physical fitness, and biomarkers in postmenopausal women with metabolic syndrome.

METHODS

Research Design

Subjects were advised for fasting for 12 h and refrained from drinking water for 2 h before testing. Subjects were asked not to engage in any physical activity for 24 h before testing. Upon arrival, BP, fasting blood glucose, glycated hemoglobin (HbA1c), anthropometrics, and body composition via electrical bio-impedance were first measured. Then, all physical tests were performed in a randomized order as follows: handgrip strength (dominant arm), timed-up-and-go (TUG), 30-s chair stand, arm curl, and sit-to-stand.

Patients

A total of 75 postmenopausal women with metabolic syndrome participated in this study (Table 1). The National Cholesterol Education Program criteria were used to diagnose metabolic syndrome[14]. During the experimental procedures, patients remained on their typical diet, avoiding any nutritional supplementation. All subjects read and signed an informed consent in accordance with the Declaration of Helsinki. Patients who had any functional limitations or medical conditions that could be aggravated by the tests were excluded. The study protocol was approved by the local ethics committee (CAE: 23081213.6.0000.5269).

Anthropometry and body composition

Body composition was measured via octopolar electrical impedance (InBody 720, Biospace, Seoul, South Korea), previously validated[15,16]. Body mass (BM), fat mass (FM), lean body mass, fat percentage, fat-free mass (FFM), and visceral fat area (VFA) were measured. Body height was measured with a stadiometer (Stadiometer Seca 208 Bodymeter), and waist, iliac, abdominal, and hip circumferences were measured with an anthropometric tape.

Table 1. Patient characteristics (mean ± SD).

	Mean ± SD	95% Confidence Interval	
		Lower	Upper
Age (years)	57.9 ± 11.14	55.3	60.4
Height (cm)	157.6 ± 6.79	156.0	159.1
Body mass (kg)	76.7 ± 15.17	73.2	80.2
Abdominal circumference (cm)	96.4 ± 11.90	93.7	99.1
Waist-hip ratio	0.9 ± 0.08	0.9	0.9
Lean body mass (kg)	23.7 ± 4.00	22.8	24.6
Fat mass (kg)	33.8 ± 10.33	31.5	36.2
Visceral fat area (cm^2)	126.4 ± 31.30	119.2	133.6
Resting metabolic rate	1304.6 ± 134.21	1273.7	1335.5
Systolic blood pressure (mmHg)	131.3 ± 23.68	125.9	136.8
Diastolic blood pressure (mmHg)	82.9 ± 12.33	80.1	85.7
Mean arterial pressure	99.1 ± 14.79	95.6	102.5
Handgrip strength	24.3 ± 6.61	22.8	25.8
Relative force	0.3 ± 0.10	0.3	0.3
Arm curl	13.2 ± 3.66	12.4	14.1
Timed-up-and-go	11.2 ± 2.22	10.7	11.8
30-s chair stand	11.0 ± 4.06	10.1	11.9
Sit and reach (cm)	19.0 ± 4.61	17.9	20.1
Glycated hemoglobin (HbA1c)	7.1 ± 1.78	6.7	7.5
Fasting glucose (mg/dl)	147.0 ± 56.51	134.0	160.0

SD: Standard deviation.

Handgrip strength

To determine muscle strength, a hydraulic grip strength dynamometer (Jamar Hydraulic Hand Dynamometer Model J00105, Lafayette Instrument Company, Inc., IN, United States) was used. During the test, each subject had three attempts with a 1-min rest interval, and the highest value was recorded. The handgrip test was performed in a seated position, with the dominant arm, with the shoulder adducted, neutrally rotated, and elbow flexed at 90°. Moreover, relative strength was calculated with the equation: handgrip strength (kg) ÷ body mass (kg).

Physical fitness test

To determine functional capacity, the TUG test was used. A previous study reported that this test could predict fall risk in older adults[17]. This test involves the time taken to rise from a chair, walk 3 m, turn around a marker, walk back to the chair, and sit down[17].

To determine upper limb strength, an arm curl test was utilized, consisting of maximal repetitions performed in 30 s[18]. During this test, subjects were seated without bending the trunk forward, and dumbbells weighing 2.3 kg were used[18]. To determine lower limb strength, a 30-s chair stand test was utilized, consisting of the maximal number of rises from a chair that could be done in 30 s. During this test, the arms were folded across the chest, and the total number of rises was recorded[19].

Blood sample analysis

Blood samples were collected after fasting for 12 h to determine fasting plasma glucose and HbA1c. Serum HbA1c concentrations were assessed by high-performance liquid chromatography, and fasting glucose was measured by using the enzymatic colorimetric method.

Statistical analysis

A series of three multiple linear regression analyses were conducted with handgrip strength as the dependent variable. The independent variables included in analyses were clinical and body composition variables (e.g., age, BM, abdominal circumferences, waist-hip ratio, body mass index [BMI], muscle mass, FM, VFA, resting metabolic rate [RMR], systolic BP [SBP], diastolic BP [DBP], mean arterial pressure [MAP]), physical fitness variables (e.g., handgrip strength, arm curl repetitions, TUG, 30-s chair stand), and biomarkers (glucose and HbA1c). The level of significance was set at $p<0.05$ for all comparisons. All statistical analyses were performed using SPSS statistical software package version 20.0 (SPSS Inc., Chicago, IL, United States).

RESULTS

Descriptive results and comparisons between body mass index classifications

The sample was stratified by BMI classification as outlined by the WHO[20]. The results of the frequency analysis showed that out of the 75 patients in the total sample, 52% (39) were classified as obese, 37.3% (28) as overweight, and 10.7% (8) as normal weight.

Relationship between handgrip strength and body composition variables, blood pressure, functional capacity, and serum glycated hemoglobin

For the first multiple linear regression analysis, handgrip strength was the dependent variable with the following independent variables: body composition, waist–hip ratio, FM, VFM, and FFM; SBP, DBP, and mean BP; and age. According to the study by Pestana and Gageiro[21], this model revealed a significant variance of $p=0.002$ and a moderate correlation of $R=0.59$[21]. The coefficient of determination ($R^2=0.35$) showed that 35% of handgrip strength variability could be explained by the independent variables. Then, the stepwise method revealed that mean BP and FFM showed the greatest regression coefficient, coefficient of determination, and significance (ANOVA; $F=9.138$ and $p=0.002$, and the regression values are $R=0.450$ and $R^2=0.202$, respectively; see Figure 1).

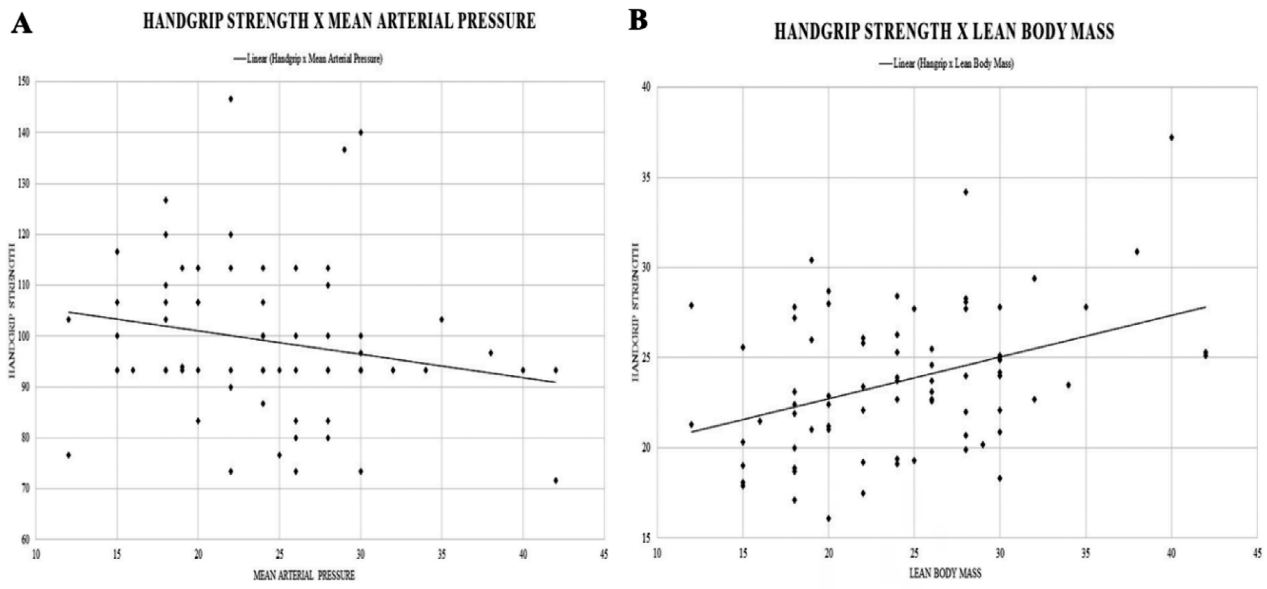
In the second multiple linear regression analysis, handgrip strength was the dependent variable with the following independent variables: relative handgrip strength, arm curl repetitions, TUG, and 30-s chair stand. According to the study by Pestana and Gageiro[21], this model revealed a significant variance of $p=0.001$ and a strong correlation of $R=0.80$. The coefficient of determination ($R^2=0.65$) showed that 65% of the handgrip strength variability could be explained by the independent variables. Then, the stepwise method revealed that arm curl repetitions showed the greatest regression coefficient, coefficient of determination, and significance (ANOVA; $F=61.938$ and $p=0.001$, and the regression values are $R=0.795$ and $R^2=0.632$, respectively; see Figure 2).

In the third multiple linear regression analysis, handgrip strength was the dependent variable with the following independent variables: handgrip strength and HbA1c and fasting glucose. According to the study by Pestana and Gageiro[21], no significant results were observed ($p=0.978$) with a weak correlation ($R=0.02$).

DISCUSSION

The key findings of the present study showed that 52% of the total sample could be classified as obese, 37.3% as overweight, and 10.7% as normal weight. In addition, based on linear regression analysis, handgrip strength was predictive of the following variables: muscle mass, MAP, and arm curl repetitions.

Steffl et al.[22] examined the association between handgrip strength, muscle mass, and physical performance in 69 community-dwelling elderly women. The results showed a high association between variables and concluded that muscle mass and handgrip strength were more predictive of sarcopenia than physical fitness status. In the present study, the first linear regression showed that handgrip strength was predictive of FFM. In a literature review, Bohannon[23] concluded that handgrip strength was associated with the presence of metabolic syndrome and



A: Handgrip strength and lean body mass; B: Handgrip strength and mean arterial pressure.

Figure 1. The regression results between variables.

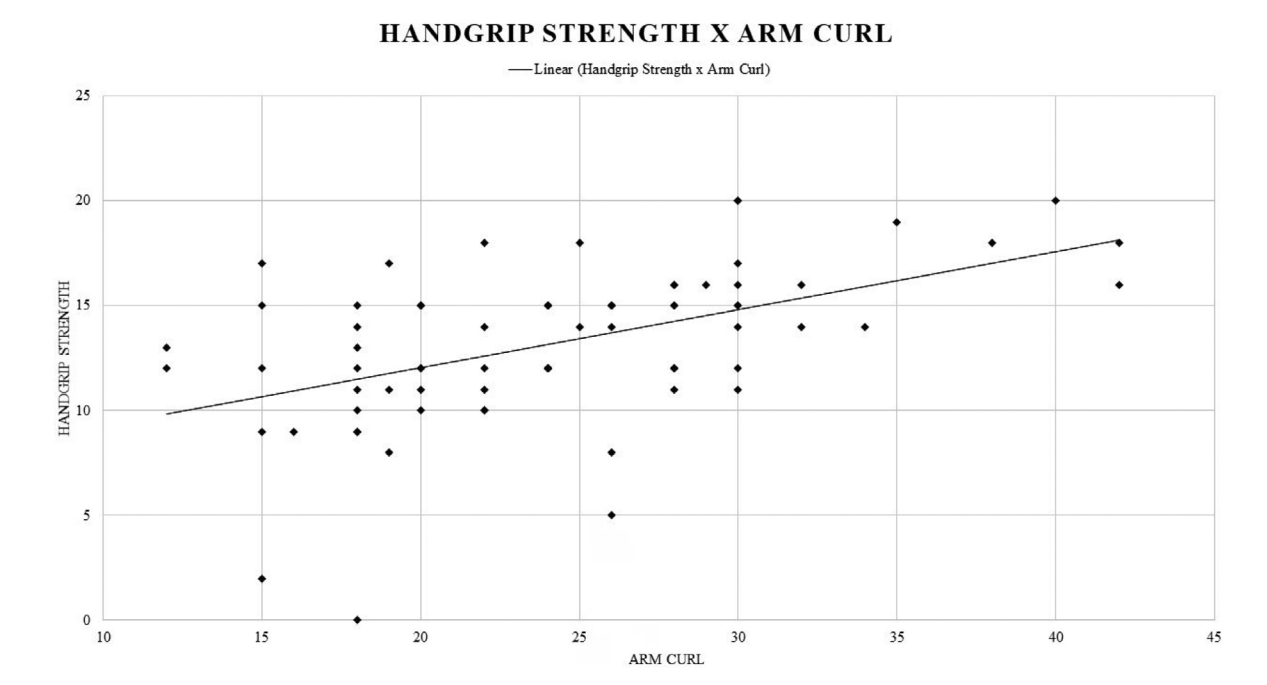


Figure 2. Handgrip strength and arm curl regression results.

was predictive of mobility and mortality. The mechanism to explain these associations might be connected to adipose tissue-releasing adipocytokines that promote chronic systemic and regional inflammation, related to poorer functional capacity, and reduce muscle strength[24].

Furthermore, in the present study, mean BP showed an inverse association with the handgrip strength based on the first linear regression analysis. Mainous et al.[25] studied the association between handgrip strength, diabetes, and hypertension. The results showed that patients with hypertension had lower

levels of handgrip strength when compared to healthy patients. Our results showed an inverse association between mean BP and handgrip strength; this result has clinical relevance for controlling high BP. Besides that, general strength training is a key strategy to prevent hypertension, including isometric handgrip training as reported in the literature[26].

In the second linear regression analysis, only one independent variable, the arm curl test, was associated with handgrip strength. Jeoung and Lee[27] studied the association between frailty and physical performance in 114 elderly women. The results showed that functional and physical fitness tests, mainly the handgrip strength test, in elderly women could be used to predict the risk of weakness. As the handgrip strength test is easy to administer, it can easily be applied in clinical settings in an elderly population. In the third linear regression analysis, there was no significant association between HbA1c and fasting glucose with the handgrip strength.

In this study, body composition was evaluated based on BMI, which can be considered a methodological limitation and future studies should use dual-energy X-ray absorptiometry or other assessments to determine overweight and obese subjects. However, for a large sample, BMI is a good and easy strategy to determine body composition and the strength of this method is the practical application. Moreover, supplementary studies are essential to improve workout intervention programs and approaches to avoid the elderly from becoming frail and to promote their health.

In conclusion, this study showed that handgrip strength could predict lean body mass, uncontrolled BP, and upper limb strength. The handgrip strength is easy to assess and could be applied to prevent early weakness and diseases like metabolic syndrome, diabetes, and hypertension.

AUTHOR'S CONTRIBUTIONS

HM: Conceptualization, Project administration, Visualization, Methodology, Writing – review & editing. **CB:** Conceptualization, Project administration, Visualization, Data curation, Formal Analysis, Methodology, Writing – review & editing. **MR:** Conceptualization, Funding acquisition, Supervision, and Visualization. **CCN:** Conceptualization, Funding acquisition, Supervision, and Visualization. **IN:** Visualization, Writing– review & editing. **JW:** Visualization, Writing– review & editing. **LM:** Conceptualization, Funding acquisition, Supervision, and Visualization.

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ORIGINAL ARTICLE

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Follow-up of a cohort of patients with noncystic fibrosis bronchiectasis for 1 year

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SUMMARY

OBJECTIVE: The objective of this study was to evaluate the quality of life of patients with noncystic fibrosis bronchiectasis during a 1-year follow-up by using the EuroQol – 5 Dimensions – 3 Levels (EQ-5D-3L) questionnaire.

METHODS: A cohort study was conducted with 100 patients with noncystic fibrosis bronchiectasis and followed up with face-to-face visits or by telephone contact every 3 months for 1 year. All patients were recruited from a single referral center for bronchiectasis. At the time of recruiting and at the end of 1 year, the EQ-5D-3L questionnaire was applied to evaluate the patients' quality of life. Variables, such as exacerbation, emergency care, comorbidities, hemoptysis, colonization, and hospitalization, were assessed.

RESULTS: Of the 100 patients, 99 completed the study and 72% were women. There were no marked limitations in the mobility and self-care domains during the follow-up. At the end of the follow-up, 32 patients were extremely anxious or depressed. The quality of life assessed by using EQ-5D-3L had an initial mean score of 0.545 and of 0.589 after 1 year, which was statistically significant (p=0.011).

CONCLUSION: Patients with noncystic fibrosis bronchiectasis have a poor quality of life, and the EQ-5D-3L questionnaire may be a tool for monitoring patients with bronchiectasis.

KEYWORDS: Bronchiectasis. Quality of life. EQ-5D-3L. Exacerbation. Hospitalization.

INTRODUCTION

Noncystic fibrosis bronchiectasis (NCFB) is an irreversible disease, characterized by bronchial dilatation[1,2] resulting from the destruction of the elastic and muscular components of its walls[3]. In general, symptoms include a chronic cough, sputum with or without hemoptysis, dyspnea, intermittent respiratory infections[4], and fatigue[5]. Exacerbations are frequently observed in most bronchiectasis patients and have been associated with progressive loss of lung function, worsening of quality of life[3,4], and increased mortality[2,5]. These disorders lead to changes in a patient's daily life and may restrict their usual activities. The worldwide epidemiological situation is unknown and varies according to the demographic area. The prevalence of NCFB in the American population is estimated at 139 cases per 100,000 individuals, with an annual incidence of 29 cases per 100,000 Americans[6]. In Germany, the estimated proportion is 67 per 100,000 inhabitants[4]. It is estimated that the prevalence and incidence of bronchiectasis in Brazilian individuals are high because they are mainly related to pulmonary tuberculosis (TB) that is highly prevalent in Brazil (coefficient of incidence of 33.5 cases per 100,000 inhabitants in 2017)[7] and results in bronchiectasis sequelae in many patients[8]. NCFB is also associated with inadequate control of respiratory infections during childhood, difficulty in accessing health resources, and low socioeconomic status[9,10]. There are several instruments that can be used to evaluate the health-related quality of life (HRQoL) in patients affected by numerous diseases. Among them is the EuroQol – 5 Dimensions – 3 Levels (EQ-5D-3L)[11], a simple, easy-to-understand, widely used instrument, available in multiple languages and with various modes of administration. The EQ-5D-3L questionnaire addresses five important dimensions or domains related to patient health. Measuring the quality of life can help guide individualized treatment and contribute to better care. Bronchiectasis is a disease characterized by a high morbidity and mortality[12,13], and studies are needed to better understand the evolution of the disease and improve patient care. The objective of this study was to evaluate the quality of life of patients with bronchiectasis during a 1-year follow-up by using the EQ-5D-3L questionnaire.

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METHODS

Subject and study design

A cohort study was carried out in a university hospital in the State of Rio de Janeiro, from January 2017 to May 2018. Patients older than 18 years were recruited from an outpatient clinic specialized in pulmonology. All patients underwent high-resolution computed tomography (HRCT), which is considered the gold standard for the diagnosis of bronchiectasis. The Research Ethics Committee of the University Hospital Pedro Ernesto, Brazil, approved the research (no. 1,823,665). The patients were individually interviewed, a structured questionnaire with demographic and clinical data was administered. Then, the Quality-of-Life Questionnaire (EQ-5D-3L) and the pulmonary function test were scheduled. During the 12-month follow-up, the interviews were conducted by telephone or face-to-face contact before or after medical appointments, with an interval of 3 months. At the end of 1 year, the patients underwent a new spirometric test and responded to the EQ-5D-3L and Modified Medical Research Council (mMRC) questionnaires.

Outcome data

There are several factors that favor the development of bronchiectasis[1,9,10]. In this study, we considered the following groups: idiopathic, postinfectious by pulmonary TB, postinfection non-TB, primary immunodeficiency (common variable immunodeficiency), Kartagener's syndrome, and "undetermined." The cases in which the etiology was under investigation or was incomplete were classified as "undetermined" etiology.

Exacerbation was defined as the care of the patient in an outpatient unit when not previously scheduled or in an emergency unit, with or without the need for antibiotic therapy intervention, with the at least three of the following four clinical data: increased dyspnea intensity, increased daily volume of sputum, altered secretion color, or fever [4,14] (>37.5°C). According to the study by Murray et al., sputum was defined as mucoid (clear), mucopurulent (pale yellow/pale green), and purulent (dark yellow/dark green)[15].

Patients who had a daily cough with a mucoid, mucopurulent, or purulent sputum for at least 3 consecutive months in the 12-month period were considered as having "wet" bronchiectasis.

Functional indices such as the pre- and post-bronchodilator forced expiratory volume in 1 s (FEV_1), forced vital capacity (FVC), and FEV_1/FVC ratio were evaluated at the initial consultation and after 12 months. Ventilatory disorders were defined according to the criteria published by the American Thoracic Society (ATS)/European Respiratory Society (ERS) as normal, obstructive, restrictive, and mixed[16].

Baseline and follow-up questionnaire

The baseline questionnaire contained the following data for collection: age, body mass index (BMI), the number of exacerbations, emergency visits, hospitalizations, the presence of fever (>37.5°C), increased dyspnea and sputum, change in sputum color and appearance, hemoptysis, the degree of dyspnea, the mMRC, therapeutic intervention with antibiotics, smoking (active, passive, ex-smokers, and nonsmokers), spirometry, etiology, "wet bronchiectasis," the number of affected lobes (the lingula was considered a separate lobe), daily approximate volume of sputum, comorbidities, vaccines (e.g., influenza and pneumococcal), respiratory physical therapy, colonization with *Pseudomonas aeruginosa* (PA), *Aspergillus*, and infections caused by nontuberculous mycobacteria (NTM). Items monitored at follow-up included the number of exacerbations, emergency visits, hospitalizations, the presence of fever (>37.5°C), increased dyspnea and sputum, change in sputum color and appearance, hemoptysis, and antibiotic therapy.

The quality-of-life questionnaire is an instrument composed of the EQ-5D-3L questionnaire and the Visual Analogue Scale (VAS)[11]. The EQ-5D-3L jointly addresses physical functions (i.e., mobility, self-care, and pain/discomfort domains), social functions (i.e., habitual activities domain), and mental functions (i.e., anxiety/depression domain). Each domain/dimension is related to three levels of severity (i.e., no problems, some problems, and extreme problems)[11]. The VAS consists of a ruler numbered from zero (worst health state imaginable) to 100 (best health state imaginable)[11], and the patient records the value that best represents his or her health state at the time. The survey was registered in the EuroQol Research Foundation website, and an authorization to apply the self-complete version of the EQ-5D-3L questionnaire and the face-to-face version of the EQ-5D-3L for patients with reading or writing difficulties was obtained. A validated version of the questionnaire in Portuguese was used in the study[11].

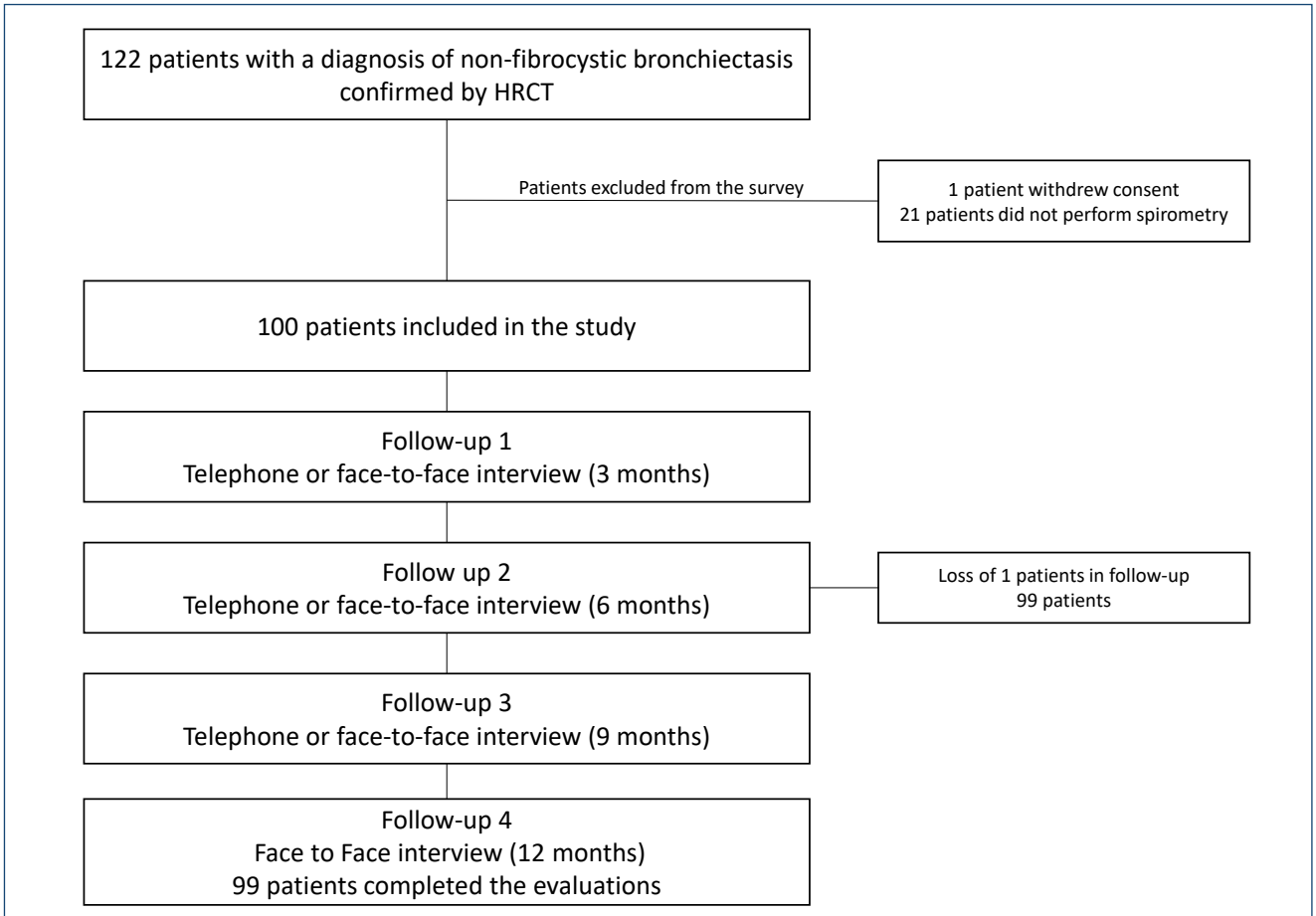
For the sample calculation, a standard deviation of 0.5 was considered, requiring 50 patients to detect a 7% difference in the quality of life with 95% confidence and 80% power. Numerical data were presented using mean and standard deviation or median and interquartile range, and categorical data were presented using percentage and absolute values. The Student's t, Mann–Whitney U, Kruskal–Wallis, ANOVA, chi-square, and Fisher's exact tests were used. For the elaboration of the graphs, the plotly package was used.

RESULTS

Of the 122 patients recruited, 1 patient withdrew the consent form, 21 were excluded because they did not meet all the eligible criteria, and 1 patient was lost to follow-up. Finally, only 100 patients were included in the analysis (Figure 1).

The general characteristics of the study population are expressed in Table 1. The patients were predominantly female (72%) and nonsmokers (81%), with a mean age of 56.94±15.32 years and a mean BMI of 24.42±5.13 kg/m^2. Women were older and had a higher BMI than men. In the tomographic findings, 79 (79%) individuals had bronchiectasis in more than one pulmonary lobe. The etiological predominance was related to the sequelae of pulmonary TB in 53% of the cases. Of the 79 (79%) patients with an associated comorbidity, 29 (29%) had at least one aggravation and 50 (50%) had two or more associated aggravations. Based on the history of recurrent respiratory disease, 55 (55%) patients had rhinosinusitis and 53 (53%) had a previous pneumonia at least once. The most commonly reported comorbidities in descending order were as follows: systemic arterial hypertension (SAH, 27.9%), chronic obstructive pulmonary disease (COPD, 25.3%), diabetes mellitus (DM, 21.5%), and osteoarticular diseases (16.5%).

In the course of 1 year, 21 (21%) patients were colonized by PA and 2 (2%) were colonized by NTM. Also, 3 (3%) patients were previously colonized by fungi (*Aspergillus*). In the beginning of the study, 21 (21%) subjects presented grade 3 dyspnea according to the mMRC scale, and after 12 months of follow-up, this categorization increased by 53% (32 subjects). In addition, 22 (22%) patients had an exacerbation, 27 (27%) had two or more exacerbations, and 50 (50%) experienced no exacerbations. Of the 49 (49%) individuals who experienced exacerbations, 5 required hospitalization and 1 patient required two hospitalizations. A total of 20 (20%) patients sought the emergency unit or went to the clinic without prior appointment at least once, while 34 (34%) sought care two or more times (Table 2). There were no deaths during the study. Therapeutic intervention with antibiotics was not necessary in more than 50% of the patients.



HRCT = High-resolution computed tomography. Follow-up 1, 2, 3, and 4 = monitoring the number of exacerbations, emergency visits, hospitalization, presence of fever (>37.5°C), increased dyspnea and sputum, altered color and sputum appearance, hemoptysis, and antibiotic therapy.

Figure 1. Flowchart of the population in the study.

Table 1. Demographics and clinical characteristics of patients with noncystic fibrosis bronchiectasis.

Data	n=100	Male n=28	Female n=72	p-value*
Age, years	56.94±15.32	55±15.31	57.69±15.36	0.435
Body mass index, kg/m²	24.42±5.13	22.75±3.44	25.07±5.54	0.042
Smoking status				
Current smoker	4 (4.0)	1 (3.6)	3 (4.2)	
Former smoker	12 (12.0)	6 (21.4)	6 (8.3)	0.2498
Passive smoker	3 (3.0)	0	3 (4.2)	
Nonsmoker	81 (81.0)	21 (75.0)	60 (83.3)	
Etiology				
Idiopathic	15 (15.0)	4 (14.2)	11 (15.3)	
Postinfection tuberculosis	53 (53.0)	16 (57.2)	37 (51.4)	
Postinfection nontuberculosis	23 (23.0)	7 (25.0)	16 (22.2)	0.6403
Kartagener's syndrome	2 (2.0)	1 (3.6)	1 (1.4)	
Primary immunodeficiency	1 (1.0)	0	1 (1.4)	
Undetermined*	6 (6.0)	0	6 (8.3)	
Moist bronchiectasis	44 (44.0)	13 (46.4)	31 (43.1)	0.8243
Number of affected lobes, n=92				0.5023
1 lobe	13 (14.1)	2 (8.0)	11 (16.4)	
≥2 lobes	79 (85.9)	23 (92.0)	56 (83.6)	
Approximate daily sputum volume				0.4080
<100 ml	47 (47.0)	16 (57.2)	31 (43.1)	
100–200 ml	23 (23.0)	6 (21.4)	17 (23.6)	
No sputum	30 (30.0)	6 (21.4)	24 (33.3)	
Number of comorbidities				0.8020
No comorbidities	21 (21.0)	7 (25.0)	14 (19.5)	
1 comorbidity	29 (29.0)	8 (28.6)	21 (29.2)	
≥2 comorbidities	50 (50.0)	13 (46.4)	37 (51.4)	
Previous respiratory disease				
Rhinosinusitis	55 (55.0)	16 (57.1)	39 (54.2)	0.8262
Tuberculosis	55 (55.0)	18 (64.9)	37 (51.9)	0.2710
Pneumonia	53 (53.0)	12 (42.9)	41 (56.9)	0.2656
Comorbidities	n=79	n=21	n=58	
Systemic arterial hypertension	45 (57.0)	8 (38.1)	37 (63.8)	0.0704
Diabetes mellitus	17 (21.5)	5 (23.8)	12 (20.7)	0.7637
GERD	7 (8.9)	1 (4.8)	6 (10.3)	0.6680
Osteoarticular disease	13 (16.5)	1 (4.8)	12 (20.7)	0.1666
Neoplastic disease	8 (10.1)	3 (14.3)	5 (8.6)	0.4322
Cardiovascular disease	6 (7.6)	2 (9.5)	4 (6.9)	0.6538
HIV	2 (2.5)	1 (4.8)	1 (1.7)	0.4635
COPD	20 (25.3)	7 (33.3)	13 (22.4)	0.3836
Asthma*	22 (27.9)	7 (33.3)	15 (25.9)	0.5744
Pneumonectomy	3 (3.8)	0	3 (5.2)	0.5610
Lobectomy	5 (6.3)	3 (14.3)	2 (3.4)	0.1139
Depressive disorder	7 (8.9)	1 (4.8)	6 (10.3)	0.6696
Hypothyroidism	4 (5.1)	0	4 (6.9)	0.5687
Other comorbidities*	21 (26.6)	6 (28.6)	15 (25.9)	0.7816

Date are presented as number (%) or mean±standard deviation (SD). BMI: body mass index; GERD: gastroesophageal reflux disease; COPD: chronic obstructive pulmonary disease; HIV: human immunodeficiency virus. *Other comorbidities such as psoriasis, chronic renal failure, alpha-1 anti-trypsin deficiency, cor pulmonale, glaucoma, Sjögren syndrome, and malnutrition.

The presence of limitations in relation to domains is detailed in Table 3. It was observed that no patient was confined in bed (mobility domain, level 3) or was unable to maintain their personal care (self-care domain, level 3) between the onset and after 12 months of follow-up. The habitual activities and pain/discomfort domains were statistically significant (p=0.0078 and p=0.0097, respectively). An increase of 18.5% in the incidence of extreme anxiety/depression was observed. During the study, no patient presented extreme limitations in all the domains. The quality-of-life assessment had an average score of 0.545±0.187 and 0.589±0.208, respectively (Table 3), between the onset and after the 1-year follow-up**.** There was statistical significance in the evaluation of HRQoL determined by EQ-5D-3L (p=0.011) and VAS (p=0.0018) (Table 3). After 1 year, 3 patients were unable to undergo a new spirometric test due to the associated diseases. Pulmonary function assessments showed a predominance of an obstructive ventilatory disorder (OVD) at baseline (67%) and after 12 months (72.9%).

Table 3 presents the mean value of the quality of life stratified by groups: with exacerbation and without exacerbation, with emergency unit care and those who did not require emergency care, with comorbidities and without comorbidities, and with hemoptysis and without hemoptysis. There was a statistically significant difference at the beginning and after 1 year of follow-up between the group with and without exacerbation (p=0.002 and p=0.001, respectively) and between the group with and without emergency unit care (p=0.006 and p=0.011, respectively).

DISCUSSION

Bronchiectasis is a complex and heterogeneous disease with clinical, radiological, microbiological, and prognostic variability, as seen in this study. The identification of risk factors related to worsening health can help in the development of

Table 2. EQ-5D-3L and spirometry at baseline and after 1-year follow-up.

Data		Baseline n=100	1 year n=99	p-value
EQ-5D-3L	–	0.545±0.187	0.589±0.208	0.011
Domain	Level response			
Mobility	No problems	38 (38.0)	47 (47.5)	0.1985
	Some problems	62 (62.0)	52 (52.5)	
	Extreme problems	0 (0)	0 (0)	
Self-care	No problems	61 (61.0)	69 (69.7)	0.2339
	Some problems	39 (39.0)	30 (30.3)	
	Extreme problems	0 (0)	0 (0)	
Usual activities	No problems	28 (28.0)	32 (32.3)	0.0078
	Some problems	71 (71.0)	57 (57.6)	
	Extreme problems	1 (1.0)	10 (10.1)	
Pain/discomfort	No problems	18 (18.0)	36 (36.4)	0.0097
	Some problems	63 (63.0)	52 (52.5)	
	Extreme problems	19 (19.0)	11 (11.1)	
Anxiety/depression	No problems	22 (22.0)	29 (29.3)	0.2014
	Some problems	51 (51.0)	38 (38.4)	
	Extreme problems	27 (27.0)	32 (32.3)	
FEV_1, L	Pre-BD	1.35±0.60	1.32±0.61	0.1099
	Post-BD	1.38±0.61	1.37±0.62	0.3555
FVC, L	Pre-BD	2.34±2.93	2.05±0.78	0.4013
	Post-BD	2.07±0.73	2.09±0.80	0.6854
FEV_1/FVC, %	Pre-BD	64.36±13.40	64.16±13.73	0.9310
	Post-BD	1.35±0.60	1.32±0.61	0.1099

Data are expressed as n (%) and mean±SD. VAS: Visual Analogue Scale; VEF_1: forced expiratory volume in 1 s; FVC: forced vital capacity; pre-BD: pre-bronchodilation; post-BD: post-bronchodilation.

Table 3. Quality of life stratified by groups at baseline and after 1-year follow-up.

Groups	EQ-5D-3L Baseline	p-value[a]	EQ-5D-3L 1 year	p-value[b]
Exacerbation[1]	0.487±0.139	0.002	0.521±0.190	0.001
Nonexacerbation	0.601±0.210		0.656±0.204	
Visit to emergency unit[2]	0.499±0.158	0.006	0.551±0.195	0.011
Number of visits to the emergency unit	0.599±0.204		0.651±0.200	
One comorbidity[3]	0.564±0.161	0.147	0.632±0.199	0.050
≥2 comorbidities	0.511±0.196		0.539±0.207	
No comorbidities	0.601±0.189		0.650±0.200	
Hemoptysis[4]	0.537±0.169	0.768	0.576±0.204	0.664
No hemoptysis	0.549±0.195		0.595±0.211	
Colonized[5]	0.463±0.155	0.165	0.508±0.181	0.112
Noncolonized	0.555±0.189		0.605±0.211	
Hospitalized due to respiratory problems[6]	0.387±0.182	0.181	0.405±0.165	0.461
Hospitalized due to other causes	–		0.396±0.111	
Not hospitalized	0.550±0.186		0.601±0.207	

Data are expressed as mean±SD. [1]Exacerbation vs. nonexacerbation groups. [2]Visit to emergency unit vs. no visit to the emergency unit groups. [3]1 comorbidity vs. ≥2 comorbidities vs. non-comorbidities groups. [4]Hemoptysis vs. nonhemoptysis groups. [5]Colonized vs. noncolonized groups. [6]Hospitalized due to respiratory problems vs. hospitalized due to other causes vs. not hospitalized groups. [a]p=baseline. [b]p=1-year follow-up.

individualized strategies to improve the quality of life. Choosing the EQ-5D-3L multiparameter instrument to monitor the quality of life of patients facilitates understanding, as it uses a scale with score ranging from 0 to 100%. The health status of each individual was determined through the EQ-5D-3L tool. Similar to the previous findings by Hill et al.[1], Aksamit et al.[17], and Bogossian et al.[9], we found a higher incidence of bronchiectasis in women (72%) than in men (28%). The results also showed that the main etiology of bronchiectasis was related to the sequelae of pulmonary TB (52%). This cause is common in countries with a high number of TB cases[18]. This prevalence is also evident in Brazil (Bogossian et al.[9], 42.7%) and China (Xu et al.[19], 31.1%), in contrast to the etiological findings of developed countries with about 40% of cases attributable to idiopathic bronchiectasis[14]. We noted a poor quality of life for these individuals, as assessed by the EQ-5D-3L questionnaire, which is used to measure and evaluate the health status of patients with bronchiectasis. The mobility and self-care domains were associated with a lesser effect on health status, whereas habitual activities and anxiety/depression contributed to a poorer health status, thus reducing the quality of life. The study also reported that exacerbation, emergency care, comorbidities, colonization, and hospitalization had had a significant negative effect on health status. The results indicate that these variables contribute to the worsening of the quality of life of these individuals. Patients with bronchiectasis tend to present with exacerbation frequently, resulting in the aggravation or appearance of clinical symptoms that may require hospitalization and inclusion of drug therapy involving antibiotics, corticosteroids, and bronchodilators. According to the study by Redondo et al.[4], the increase in the frequency of exacerbations was associated with factors such as colonization, mainly by PA, air pollution, and comorbidities. Chang and Bilton[20] reported in their study that exacerbations result in lung function decline, a worsened quality of life, and hospital admissions. In our study, we found that exacerbations were frequent at the 1-year follow-up. Of the 49 patients who experienced exacerbations, 82% had comorbidities associated with bronchiectasis. Most exacerbations were marked by increased dyspnea and volume of sputum, sputum purulence, hemoptysis, and fever.

Currently, despite the technological advances and the elaboration of guidelines, there are still difficulties and a lack of consensus in some of the approaches related to bronchiectasis. Less is known about the real risk factors that could lead to hospitalization. Ringshausen et al.[21] reported that the average annual hospitalizations in the United States was 16.5 admissions per 100,000 inhabitants, while in Germany this value was 9.4. In our study, there were 9 (9%) hospitalizations during the follow-up. Notably, 5 individuals required hospitalization due to bronchiectasis, and 1 patient required a new hospitalization.

Hemoptysis is another complication that can affect bronchiectasis patients and may require immediate medical assistance and the administration of large blood volumes. In their recent study, Bhalla et al.[22] described the main etiologies of hemoptysis in patients who sought emergency care in India. Of the patients admitted, 65% had hemoptysis due to active pulmonary TB or its sequelae and 9.3% of cases were due to bronchiectasis. Lundgren et al.[23] who conducted their study in Brazil found that 38% of cases of hemoptysis were caused by bronchiectasis. In this study, of the 31 patients who reported small volumes of hemoptysis (<100 ml/24 h) or blood streaks, 17 previously had pulmonary TB.

Bronchiectasis individuals are commonly colonized by the pathogens PA and *Haemophilus influenzae*. In this study, 21 (21%) patients were colonized by PA. Colonization by PA may involve 25–58% of cases and tends to lead to a more rapid lung function decline, frequent exacerbations, and poorer quality of life[24].

We identified 79 patients with comorbidities concurrent with bronchiectasis, with half of the subjects presenting multiple comorbidities. The recent consensus stated that cardiovascular disorders, COPD, diabetes, gastroesophageal reflux disease (GERD), psychological diseases, and pulmonary hypertension are more likely to exist in conjunction with bronchiectasis and that such comorbidities contribute to morbidity and mortality, and worsening of the quality of life[25].

The main limitations of this study are related to sample size and the follow-up period, with long-term studies being required. Another limitation of the study was the recruitment of volunteers from a single-center specialized in bronchiectasis, representing a subgroup of patients who regularly attended medical appointments. However, one of the strengths of the article was its prospective design, with close monitoring and determining that quality of life needs to be incorporated as one of the indicators of therapeutic management.

CONCLUSION

Our population had a high frequency of exacerbations, multiple comorbidities, and airflow obstruction. Patients with NCFB presented with marked impairment in HRQoL with moderate-to-extreme limitations of their daily activities. The quality of life tended to worsen in the presence of exacerbations and in individuals who sought emergency care and had comorbidities, colonization, and hospitalization. The incorporation of a quality-of-life assessment in patients with bronchiectasis in the clinical practice is a necessary effort to be implemented, considering that this parameter may lead to an individualized treatment, thus improving the outcomes for these patients.

AUTHORS' CONTRIBUTIONS

SPM, BRT, RR: Conceptualization and writing – original draft. **SPM, CHC, AJL, CHC, BRT:** Data curation. **SPM, REBS, WC:** Investigation. **SPM, BRT, CHC, RR:** Methodology. **BRT:** Formal analysis. **SPM, RR:** Writing – review and editing, and project administration. **RR:** Supervision.

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ORIGINAL ARTICLE

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Anxiety, attitudes-behaviors, coping styles, and social support among high-risk pregnant women in the late period of the coronavirus disease 2019 pandemic

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SUMMARY

OBJECTIVE: The purpose of this study was to investigate the prevalence of anxiety among high-risk pregnant women in the late period of the coronavirus disease 2019 pandemic and to evaluate the relationship between anxiety levels, attitudes-behaviors, coping styles, and other psychometric parameters.

METHODS: Pregnant women who were followed up in our gynecology outpatient clinic were evaluated during their admissions between November 15, 2020 and February 15, 2021. This cross-sectional study analyzes prospectively collected data from a university hospital. Inclusion criteria were those being at risk of pregnancy and between the age of 18 and 45 years, while exclusion criteria were mental retardation and the presence of serious psychiatric illness. The study included 140 participants. Sociodemographic and pregnant attitudes-behaviors data form, State-Trait Anxiety Inventory, Coping Styles Scale Brief Form (Brief-COPE), and Multidimensional Scale of Perceived Social Support were used to collect data.

RESULTS: Participants had high anxiety levels (State-STAI: 40.32±9.88; Trait-STAI: 42.71±7.32) and high prevalence of probable clinical anxiety [State-STAI: 84 (60.0%); Trait-STAI: 92 (65.7%)]. The fact concerning the transmission of the coronavirus disease 2019 to the baby during pregnancy/birth, extent to which coronavirus disease 2019 pandemic prevents regular pregnancy checkups, and family subgroup-Multidimensional Scale of Perceived Social Support predicted state probable clinical anxiety. Use of disinfectants predicted trait probable clinical anxiety. Employment status predicted state/trait probable clinical anxiety. The existence of trait probable clinical anxiety was significantly associated with behavioral disengagement and substance use which are considered ineffective coping styles. Participants without trait probable clinical anxiety had significantly more adopted positive reinterpretation, one of emotion-focused coping styles.

CONCLUSION: Based on our results, the concern of the transmission of the coronavirus disease 2019 to the babies during pregnancy/birth may be the main factor influencing anxiety among high-risk pregnant women.

KEYWORDS: Anxiety. COVID-19. Social support. Pregnant women.

INTRODUCTION

Turkey was facing a new wave of the coronavirus disease 2019 (COVID-19) pandemic, which is a worldwide health disaster. In Turkey, there were 33.198 new cases and 22.0375 new active cases daily during the time period from November 15, 2020 to February 15, 2021[1].

The secondary effects of COVID-19 on the general population, such as worsening financial circumstances, quarantine conditions, and psychological reactions in emergency situations, could have a number of negative psychiatric implications[2]. According to a recent review, up to 28% of research volunteers screened in response to the COVID-19 pandemic showed symptoms of depression and anxiety[3].

Children, adolescents, geriatric population, pregnant women, and those with preexisting mental illness all require special attention as they may be disproportionately affected by the secondary psychological consequences of the pandemic. Pregnant women may face increased levels of distress due to various reasons, particularly during the period of infectious disease outbreak[4]. Prenatal anxiety, depression, and stress have been linked to negative delivery outcomes, such as miscarriage, premature labor, low birth weight, and fetal death[5]. Furthermore, research reveals that if a mother is depressed, anxious, or stressed during pregnancy, her child is more likely to have a variety of unfavorable neurodevelopmental consequences, including increased emotional, behavioral, and cognitive issues[6]. It is critical to comprehend

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the psychological effects of pandemic on pregnant women. Multiple psychological and social elements undoubtedly influence an individual's behavior and coping skills in the face of a pandemic. More objective data are urgently required at this time to offer pregnant mothers with dependable comprehensive information and psychological support[7]. Therefore, early recognition of mental health problems in pregnant women and determination of the response to these problems and coping styles, as well as the social support they have, may lead to the development of effective and comprehensive treatment programs. However, information on the impact of the COVID-19 outbreak on mental health of pregnant women is still lacking[8,9].

In this study, our aim was to measure the prevalence of anxiety among high-risk pregnant women in the late period of the COVID-19 pandemic and to evaluate the relationship between anxiety levels and attitudes-behaviors, coping styles, and other psychometric parameters.

METHODS

Participants and procedures

Between November 15, 2020 and February 15, 2021, this cross-sectional study analyzes prospectively gathered data from Sakarya University Hospital. During their admissions, pregnant women who were followed up in the gynecology outpatient clinic were evaluated. Inclusion criteria were those being at risk of pregnancy and between the age of 18 and 45 years, while the presence of mental retardation and severe psychiatric illness were the exclusion criteria. Informed consent was obtained from all pregnant women.

The study was approved by the ethical committee of our university (no. 10840098-604.01.01-E.15517).

Survey instruments

Sociodemographic and pregnant attitudes-behaviors data form was used to collect data.

The Spielberger's State-Trait Anxiety Inventory (STAI) is a two-part Likert-type scale: one part measures anxiety at a point in time (State-STAI) and another part measures overall anxiety (Trait-STAI). Each part has 20 statements, with score ranging from 20 to 80, with the higher score indicating the higher anxiety level. A cutoff score of 40 is typically used to determine if a person has probable clinical anxiety[10]. The Turkish version of Spielberger's STAI has an internal reliability of 0.94–0.96 for State-STAI and 0.83–0.87 for Trait-STAI[11,12].

The Coping Styles Scale Brief Form (Brief-COPE) measures behaviors related to stress[13] and it contains 28 statements, each describing various coping methods. These statements may be broken down into 14 subscales, each having 2 statements. Each item response is rated on a scale of 1–4. Each subscale provides a raw score that ranges from 2 to 8[14].

The Multidimensional Scale of Perceived Social Support (MSPSS) is a 12-item scale[15]. The items are scaled on a seven-point rating system, with score ranging from "definitely no" (1) to "definitely yes" (7). Family, friends, and significant others make up the three subgroups. Higher scores indicate higher perceived social support. The internal reliability coefficient of the Turkish version of the MSPSS ranged from 0.80 to 0.95 for the total score and its subgroups[16].

Statistical analysis

We conducted univariate analysis using the Mann-Whitney U-test and chi-square test in addition to descriptive statistics. A binary logistic regression analysis was performed to evaluate the association of the relevant predictors with our categorical dependent variable. Those factors showing statistical significance ($p<0.05$) were included in the regression analysis as independent variables.

RESULTS

Description of study sample

A total of 149 participants were evaluated in this study. Out of these, 9 were excluded due to missing data, and the remaining 140 were included in the study. The mean age of the participants was 29.58±5.62. Regarding educational background, 35.0% completed primary school, 40.0% completed high school, and 25.0% completed bachelor's and master's degrees. About 78.6% of the high-risk pregnant women were unemployed. The mean gestational week was 30.83±5.95. Using a five-point Likert scale, the level of knowledge about COVID-19 was 3.69±0.84, and the rate of the transmission of COVID-19 to self, family/relatives, and the baby during pregnancy/birth were 2.44±1.06, 2.41±1.09, and 2.84±1.23, respectively. In addition, the extent to which the COVID-19 pandemic prevents regular pregnancy checkups was 2.77±1.28. The main pregnancy risk factors were as follows: 17.9% of our high-risk pregnancies had gestational diabetes mellitus (GDM), 12.9% had gestational hypertension (GHT), 9.3% had twin pregnancy, 6.4% had polyhydramnios, 6.4% had pregestational DM, 5% had intrauterine growth retardation, and 5% had preterm labor. Of all individuals, 84 (60.0%) had state probable clinical anxiety and 92 (65.7%) had trait probable clinical anxiety. The most common coping style adopted by high-risk pregnant women was turning to religion

(mean >6), while substance use, behavioral disengagement, denial, and humor (mean <4) were the least adopted coping styles. Participants stated that their family provided them with the majority of their social assistance. Table 1 shows the study population's pregnancy attitudes-behaviors, anxiety levels, coping styles, and social support.

Table 1. Attitudes-behaviors, anxiety levels, coping styles, and social support levels among high-risk pregnant women.

	N (%)/mean (SD)
Overall	140 (100)
Preventive behaviors	
Use of masks	138 (98.6)
Use of disinfectants	120 (85.7)
Pay attention to social distance	135 (96.4)
Handwashing	130 (92.9)
Frequency of sleep problems during the COVID-19 pandemic	1.88 (1.20)
State-STAI score (≥40)	**40.32 (9.88)**
No	56 (40.0)
Yes	84 (60.0)
Trait-STAI score (≥40)	**42.71 (7.32)**
No	48 (34.3)
Yes	92 (65.7)
Problem-focused coping	
Using instrumental social support	5.71 (1.54)
Suppression of competing activities	5.04 (1.41)
Restraint coping	5.23 (1.35)
Planning	5.90 (1.44)
Emotional-focused coping	
Humor	3.79 (1.65)
Acceptance	5.10 (1.47)
Turning to religion	7.40 (1.11)
Positive reinterpretation	5.74 (1.31)
Using emotional social support	5.05 (1.49)
Ineffective coping	
Denial	3.74 (1.60)
Behavioral disengagement	3.73 (1.51)
Mental disengagement	4.56 (1.46)
Focus on and venting of emotions	5.19 (1.56)
Substance use	2.41 (1.14)
Multidimensional Scale of Perceived Social Support (MSPSS)	
Family	25.59 (4.35)
Friends	21.96 (6.49)
Significant other	20.31 (7.81)
Total score	67.86 (14.85)

Possible scores are between 1 and 5 (no=1, rarely=2, sometimes=3, often=4, and always=5).

Factors associated with state/trait probable clinical anxiety in the study sample

Table 2 indicates the findings of the univariate analysis for probable clinical anxiety among the participants. When evaluating the sociodemographic data, employment status was found to be significantly associated with both state and trait probable clinical anxiety.

When examining the attitudes-behaviors, state probable clinical anxiety was significantly higher in pregnant women who stated that COVID-19 pandemic more frequently prevents regular pregnancy checkups (3.01±1.30) and the concern for COVID-19 transmission to the baby during pregnancy/birth (3.12±1.19) than those who did not state such situations (2.41±1.17 and 2.43±1.17, respectively). In high-risk pregnant women, there was a significant relationship between the use of disinfectants and handwashing and trait probable clinical anxiety. The frequency of sleep problems was significantly higher in patients with trait probable clinical anxiety than those without such problems.

When examining the coping styles, , adopting behavioral disengagement and substance use was significantly higher in high-risk pregnant women with trait probable clinical anxiety than those without. Positive reinterpretation was adopted significantly high in those without trait probable clinical anxiety than in those with.

The MSPSS family subgroup scores were significantly higher in high-risk pregnant women without state probable clinical anxiety than in those with.

The concern for COVID-19 transmission to the baby during pregnancy/birth, extent to which the COVID-19 pandemic prevents regular pregnancy checkups, and MSPSS family subgroup were found to be predictors of state probable clinical anxiety. Use of disinfectants was found to be a predictor of trait probable clinical anxiety. Employment status was found to be a predictor of both state and trait probable clinical anxiety (Table 3).

DISCUSSION

Despite positive findings such as increased availability of information and assurance through healthcare professionals and primary care[17], our study found that in the late period of the COVID-19 pandemic, high-risk pregnant women experienced high levels and prevalence of anxiety. The mean State-STAI and Trait-STAI scores were above the limit for probable clinical anxiety (≥40)[10]. As a result, people with probable clinical anxiety

Table 2. The relationship between probable clinical anxiety and sociodemographic factors, attitudes-behaviors, coping styles, and social support among high-risk pregnant women.

	State-STAI<40 N (%)/mean (SD)	State-STAI≥40 N (%)/mean (SD)	p-value	Trait-STAI<40 N (%)/mean (SD)	Trait-STAI≥40 N (%)/mean (SD)	p-value
Employment status						
Employment	7 (23.3)	23 (76.7)	**<0.05***	4 (13.3)	26 (86.7)	**<0.01***
Unemployment	49 (44.5)	61 (55.5)		44 (40.0)	66 (60.0)	
Use of disinfectants						
No	6 (30.0)	14 (70.0)	0.324*	2 (10.0)	18 (90.0)	**<0.05***
Yes	50 (41.7)	70 (58.3)		46 (38.3)	74 (61.7)	
Handwashing						
No	2 (20.0)	8 (80.0)	0.315*	0 (0.0)	10 (100.0)	**<0.05***
Yes	54 (41.5)	76 (58.5)		48 (36.9)	82 (63.1)	
Frequency of sleep problems during the COVID-19 pandemic	1.63 (0.96)	2.05 (1.32)	**0.074"**	1.54 (0.82)	2.05 (1.33)	**<0.05"**
Problem-focused coping						
Using instrumental social support	5.82 (1.43)	5.64 (1.61)	0.608"	5.67 (1.56)	5.74 (1.53)	0.888"
Suppression of competing activities	5.04 (1.40)	5.05 (1.43)	0.993"	4.92 (1.38)	5.11 (1.43)	0.433"
Restraint coping	5.29 (1.17)	5.19 (1.47)	0.650"	5.3 (1.32)	5.18 (1.37)	0.596"
Planning	6.14 (1.31)	5.74 (1.50)	0.122"	6.04 (1.37)	5.83 (1.47)	0.371"
Emotional-focused coping						
Humor	3.75 (1.68)	3.81 (1.64)	0.756"	3.60 (1.66)	3.88 (1.64)	0.252"
Acceptance	5.14 (1.37)	5.07 (1.54)	0.694"	5.25 (1.44)	5.02 (1.48)	0.338"
Turning to religion	7.46 (0.93)	7.36 (1.22)	0.935"	7.58 (0.87)	7.30 (1.21)	0.231"
Positive reinterpretation	5.96 (1.31)	5.60 (1.30)	**0.070"**	6.13 (1.10)	5.54 (1.37)	**<0.05"**
Using emotional social support	5.30 (1.54)	4.89 (1.44)	0.117"	5.02 (1.55)	5.08 (1.46)	0.721"
Ineffective coping						
Denial	3.96 (1.71)	3.60 (1.52)	0.224"	3.81 (1.66)	3.71 (1.58)	0.743"
Behavioral disengagement	3.43 (1.40)	3.93 (1.56)	**0.073"**	3.29 (1.46)	3.96 (1.50)	**<0.01"**
Mental disengagement	4.70 (1.33)	4.48 (1.54)	0.238"	4.73 (1.41)	4.48 (1.49)	0.408"
Focus on and venting of emotions	4.91 (1.52)	5.37 (1.57)	0.127"	4.85 (1.49)	5.36 (1.57)	**0.090"**
Substance use	2.34 (1.01)	2.46 (1.23)	0.369"	2.15 (0.71)	2.55 (1.30)	**<0.05"**
Multidimensional Scale of Perceived Social Support (MSPSS)						
Family	26.84 (2.18)	24.75 (5.17)	**<0.05"**	26.46 (3.11)	25.13 (4.83)	0.284"
Friends	22.95 (6.35)	21.31 (6.54)	**0.082"**	22.33 (6.13)	21.77 (6.70)	0.676"
Significant other	20.41 (8.08)	20.24 (7.69)	0.807"	20.40 (7.90)	20.26 (7.80)	0.752"
Total score	70.20 (13.64)	66.30 (15.49)	0.165"	69.19 (13.61)	67.16 (15.48)	0.530"

*Chi-square test. "Mann-Whitney U-test. Bold value indicates statistically significant.

Table 3. Binary logistic regression analysis of factors associated with probable clinical anxiety of high-risk pregnant women.

	B	SE	Wald	ff	Sig.	OR	95%CI for OR
State-STAI probable clinical anxiety							
Concern for the transmission of COVID-19 to the baby during pregnancy/birth	0.460	0.164	7.883	1	**0.005**	1.583	1.149–2.182
Extent to which the COVID-19 pandemic prevents regular pregnancy check-ups	0.351	0.155	5.136	1	**0.023**	1.421	1.049–1.925
Family-MSPSS	-0.158	0.060	6.963	1	**0.008**	0.854	0.759–0.960
Employment status							
Employment (ref.)	-	-	-	-	-	-	-
Unemployment	-1.083	0.515	4.417	1	**0.036**	0.339	0.123–0.930
Trait-STAI probable clinical anxiety							
Employment status							
Employment (ref.)	-	-	-	-	-	-	-
Unemployment	-1.530	0.604	6.419	1	**0.011**	0.216	0.066–0.707
Behavioral Disengagement	0.198	0.147	1.812	1	0.178	1.218	0.914–1.624
Use of disinfectants							
No	–	–	–	–	–	–	–
Yes	-1.861	0.806	5.333	1	**0.021**	0.155	0.032–0.755
Substance use	0.346	0.263	1.728	1	0.189	1.414	0.844–2.369
Positive Reinterpretation	-0.303	0.159	3.615	1	**0.057**	0.739	0.541–1.009
Frequency of sleep problems during the COVID-19 pandemic	0.316	0.192	2.724	1	0.099	1.372	0.942–1.998

State-STAI: Nagelkerke R^2: 0.263, Hosmer and Lemeshow test: 0.663; Trait-STAI: Nagelkerke R^2: 0.279, Hosmer and Lemeshow test: 0.884. OR: odds ratio, CI: confidence interval, df: degree of freedom. Bold value indicates statistically significant.

required to be evaluated for an anxiety disorder. Hocaolu et al. who conducted the study in pregnant women during the delay period of the pandemic reported that trait anxiety was higher than state anxiety and that maternal anxiety was high[8] (State-STAI: 39.52±10.56; Trait-STAI: 42.74±8.33). In a study conducted by Yassa et al., the mean STAI-T score of 203 pregnant women was 41.96±9.15, and the incidence of STAI >40 score was 62.6%. Although our study was conducted in the late period of the pandemic, it was remarkable that our results were very close to the levels and prevalence of anxiety of the study conducted at the beginning of the pandemic (April 2020)[18].

Employment status predicted state/trait probable clinical anxiety. We think that this situation is related to the fact that unemployment reduces the risk of transmission by preventing contact with other people. According to the study conducted by Mortazavi et al. in 484 pregnant women with healthy fetuses between May 5 and August 5, 2020, the prevalence of employment (22.9%) was very close to our study and being employed predicted worry similar to our study[19]. In a study conducted by Hocaoglu et al. with 283 pregnant women during the COVID-19 pandemic, the prevalence of employment status was very close to our study (78.1%), but employment status did not predict state/trait anxiety[8].

The concern for the transmission of COVID-19 to the baby during pregnancy/birth and extent to which the COVID-19 pandemic prevents regular pregnancy checkups predicted the state probable clinical anxiety. According to Yassa et al., 42% of pregnant women thought their baby would be infected after delivery during the pandemic[20]. In a study of prenatal and postnatal women in the COVID-19 pandemic, Lee et al. reported that many participants were unclear about the likelihood of vertical COVID-19 transmission, and 81.3% stated that pregnant women were more vulnerable to COVID-19 than the general population[21]. The effects of COVID-19 on pregnant women and their children are poorly documented. Participants who used disinfectants and washed their hands

for preventing COVID-19 had less trait probable clinical anxiety. Moreover, using disinfectants predicted less trait probable clinical anxiety. Consistent with our study, in a study conducted in the general population (n=1210) in China during the early phase of the pandemic, washing hands after touching contaminated objects was associated with lower levels of anxiety[22]. We think that these behaviors may have a mitigating effect on anxiety in high-risk pregnant women during the pandemic. Trait probable clinical anxiety was higher in those who had frequent sleep problems during the pandemic. In two separate studies conducted in 1,794 and 751 pregnant women in the early period of the pandemic, decreased sleep duration and poor sleep quality were associated with increased anxiety symptoms, supporting our findings[23,24].

The existence of trait probable clinical anxiety was significantly associated with behavioral disengagement and substance use, which are considered ineffective coping styles. On the contrary, high-risk pregnant women without trait probable clinical anxiety had significantly more adopted positive reinterpretation, one of the emotion-focused coping styles. In an online study conducted in 304 pregnant women in Canada, GAD-7 anxiety scores were significantly positively correlated with dysfunctional (ineffective) coping style ($r=0.53$, $p<0.01$) and negatively correlated with emotion-focused coping style ($r=-0.12$, $p<0.05$), while it was not significantly correlated with problem-focused coping style ($r=0.10$, $p>0.05$) during the COVID-19 pandemic[25]. During the COVID-19 pandemic, Zhu et al. found that positive coping was negatively correlated with total anxiety and depression score in frontline healthcare workers[26]. In a study evaluating substance use as a coping strategy in 83 pregnant women during the COVID-19 pandemic, more substance use was associated with elevated stress, depressive symptoms, and poorer mental health[27]. These findings are consistent with the data of our study. In general, we think that problem- and emotion-focused (i.e., positive reinterpretation) coping styles should be adopted instead of ineffective coping styles (i.e., behavioral disengagement and substance use), and strengthening therapeutic approaches in this direction may be effective in reducing anxiety in pregnant women during the COVID-19 pandemic.

In the earlier stages of the COVID-19 pandemic, Lebel et al. conducted an online study in 1,987 pregnant women and found that anxiety symptoms were negatively associated with perceived social support ($r=-0.315$, $p<0.001$)[9]. In addition, in a study of 79 pregnant women with hypertension and diabetes, low social support was correlated with higher anxiety scores ($r=-0.273$, $p<0.05$)[28]. However, this study showed that total perceived social support score was not associated with anxiety levels whereas perceived social support family subgroup scores predicted state probable clinical anxiety among the participants. In this context, more family and social support in high-risk pregnant women draws attention in relation to less state probable clinical anxiety. Social support is considered to reduce the effects of prenatal maternal stress on the infant's stress reactions, thereby buffering the biological stage of stress from mother to baby[29].

The strengths of this study are that the survey data were collected through face-to-face interviews with our follow-up patients, providing comprehensive data on the sociodemographic and attitude-behavioral characteristics of the participants, and referring to probable clinical anxiety. The limitations of our study were that the measurements were carried out using self-report assessments and that it was a single-center study.

CONCLUSION

Our findings revealed that during the late period of the COVID-19 pandemic, high-risk pregnant women may suffer significant levels of anxiety symptoms and require a lot of social support. To reduce anxiety and improve attitudes-behaviors of pregnant women, information by specialists, appropriate focused coping styles (i.e., positive reinterpretation), and especially family social support could be a guide in rehabilitation of pregnant women. In addition, it is important to arrange the work environment in a way to reduce social contact in high-risk pregnant women, giving rest leave when necessary, and remedial interventions for sleep problems to combat anxiety during the pandemic.

AUTHORS' CONTRIBUTIONS

AK: Conceptualization, Formal Analysis, Investigation, Methodology, Resources, Writing – original draft. **KG:** Conceptualization, Data curation, Formal Analysis, Investigation, Methodology, Resources, Writing – original draft. **LY:** Formal Analysis, Resources, Writing – original draft. **ZK:** Conceptualization, Methodology, Resources, Supervision, Validation, Writing – review & editing. **AÖ:** Conceptualization, Data curation, Investigation, Resources, Writing – original draft. **BK**: Data curation, writing – original draft. **NT:** Investigation, Resources, Writing – original draft. **OK:** Data curation, Investigation, Resources, Writing – original draft. **MSB:** Data curation, Investigation, Methodology, Validation, Writing – review & editing. **SÖ:** Methodology, Supervision, Validation, Writing – review & editing.

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ORIGINAL ARTICLE

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Prevalence of molecular and serological tests of the new coronavirus (SARS-CoV-2) in Carlos Chagas laboratory – Sabin group in Cuiabá

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SUMMARY

BACKGROUND: Coronavirus disease 2019, which is caused by the new severe acute respiratory syndrome coronavirus 2, became a pandemic in 2020 with a mortality rate of 2% and high transmissibility, thus making studies with an epidemiological profile essential.

OBJECTIVES: The aim of this study was to characterize the population that performed the severe acute respiratory syndrome coronavirus 2 molecular and serological tests in Carlos Chagas Laboratory – Sabin Group in Cuiabá.

METHODS: A retrospective cross-sectional study was carried out with all the samples collected from nasal swab tested by RT-PCR and serological for severe acute respiratory syndrome coronavirus 2 IgM/IgG from the population served between April and December 2020.

FINDINGS: In the analysis period, 23,631 PCR-coronavirus disease 2019 examinations were registered. Of this total number of cases, 7,649 (32.37%) tested positive, while 15,982 (66.31%) did not detect viral RNA and 374 of the results as undetermined. The peak of positive RT-PCR performed in July (n=5,878), with 35.65% (n=2,096). A total of 8,884 tests were performed on serological test SOROVID-19, with a peak of 1,169 (57.16%) of the positive tests for severe acute respiratory syndrome coronavirus 2 in July.

MAIN CONCLUSIONS: Molecular positivity and serological tests, both peaked in July 2020, were mostly present in women aged 20–59 years, characterizing Cuiabá as the epicenter of the Midwest region in this period due to the high rate of transmissibility of severe acute respiratory syndrome coronavirus 2.

KEYWORDS: Coronavirus. Reverse Transcriptase PCR. Serology. Immunoglobulin G. Immunoglobulin M.

INTRODUCTION

Coronavirus disease 2019 (COVID-19), which is caused by the new severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), became an alarming threat to public health in 2020, despite global efforts to prevent its spread[1]. SARS-CoV-2 spread rapidly, reaching more than 100 countries in five continents, forcing the World Health Organization (WHO) to declare COVID-19 as a pandemic on March 11, 2020[2,3,18].

It is an RNA virus that can cause sickness in the upper and lower respiratory tracts in immunocompromised patients with chronic conditions, the elderly, and, on rare occasions, adolescents and teenagers[4]. After this contact, there is an average infection incubation period of 3–5 days following a known exposure to someone with suspected or confirmed COVID-19, with an interval of up to 12 days[5]. COVID-19 is a new disease that deserves special attention and care because the symptoms among infected people, from mild to severe, with mortality estimated at just over 2%[3].

The transmission of this virus occurs quickly through aerosols in patients undergoing airway procedures, such as orotracheal intubation or airway aspiration. Thus, some population groups are more vulnerable to being affected by the disease, due to general conditions, ranging from health conditions to the way of life to which they are exposed[6]. Patients who meet the criteria for suspected cases should be tested for SARS-CoV-2, using samples collected from the nasopharyngeal mucous by the nasal swab[6,7]. The SARS-CoV-2 RNA is detected by reverse

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transcription-polymerase chain reaction (RT-PCR) and a positive SARS-CoV-2 test confirms the diagnosis of COVID-19[8].

However, if the initial test is negative, but suspected of COVID-19, WHO recommends resampling and testing of various airway locations, as well as testing for antibodies[9]. The tests are based on the principle of lateral flow immunoassay for the detection of IgG/IgM antibodies against SARS-CoV-2 in whole blood, serum, and plasma of humans, requiring quantification according to the onset of symptoms reported by the patient to avoid false-negative results[10]. Thus, identifying the magnitude of the health problem in the population is the first step toward the development of effective decision-making strategies in evidence-based public health situations[11,12], as well as understanding the spatial distribution of the disease is fundamental for the development of strategies during the early stages of the COVID-19[13] emergency.

In this regard, among the clinical and laboratory repercussions of the patient with COVID-19, the aim of the present study was to characterize the population that performed the SARS-CoV-2 molecular and serological tests in Carlos Chagas Laboratory – Sabin Group in Cuiabá from April to December 2020.

METHODS

This is a retrospective cross-sectional study with samples from the secondary database of Carlos Chagas Laboratory – Sabin Group, in Cuiabá – MT, collected between April and July 2020. To verify the prevalence of molecular and serological tests for SARS-CoV-2, all samples from people seen in the laboratory of both sexes were included, without age restriction; and the reports of the molecular tests performed by qPCR-RT through the extraction of the genetic material of the nasal swab virus, as well as serological tests SOROVID-19 (IgM and IgG) for the detection of antibodies to SARS-CoV-2. Rapid IgG or IgM test data were excluded from the study.

Data were expressed as absolute frequency and percentages with tabulation in Microsoft Excel. The time trend and the age distribution of the COVID-19 cases detected in the study laboratory were plotted using GraphPad version 5.04 software (Windows®). Cuzik test was used to analyze the time trend across the month.

Data in tables represent the mean and respective standard deviation, with 95% confidence interval (95%CI) within tabulation.

This study was approved by the Ethics Committee of UNIVAG "Centro Universitário" under protocol number CAAE: 37320320.1.0000.5692.

RESULTS

This study results demonstrate the data between April and December 2020, and 23,631 PCR-COVID-19 tests were registered in Carlos Chagas Laboratory – Sabin Group laboratories located in Cuiabá. Out of the total number of cases, 7,649 (32.37%) tested positive, while 15,982 (66.31%) did not have the disease and 324 (1.36%) of the visits resulted in an indeterminate (data not shown) (Table 1).

In the same period, 8,884 SOROVID-19 tests (IgG and IgM) were recorded at the Carlos Chagas Laboratory – Sabin Group laboratory in the municipality of Cuiabá. In the overall cases, 1,993 (22.43%) tested positive for both tested groups and/or just IgG or IgM, with IgG being detected late in the disease progression, immunity against viruses, and IgM recent contact with viruses that the sick person transmits when in contact with other people who live together, while 6,891 (77.57%) of the tests were nonreactive (Table 2).

There was a predominance of 5,258 (59.18%) cases in women, reaching the majority between the age group of 20 and 59 years. Positive cases registered with a peak (27.48%) in July (n=2.102) totaling 5,778 examinations performed in that month for PCR-COVID-19 test, undetected (n=3,676) and undetermined (n=374; data not shown) (Table 2).

Of the total number of cases, 4,296 (56.2%) were women and 3,353 (43.8%) were men, individuals between 20 and 59 years of age were the ones who most performed the examinations in that month. In the results of July, 5,778 tests were recorded; of these, 2,102 (36.38%) were confirmed positive for PCR COVID and 3,676 (63.62%) were confirmed negative for the disease. Of the total cases in July, 3,468 (60.0%) were women and 2,310 (40.0%) were men, remaining in the same range of individuals aged 20–59 years (Table 2).

The total of 2,008 reagent tests performed by SOROVID-19 at the Carlos Chagas Laboratory – Sabin Group Laboratory experienced a significant increase in the total number of tests collected per month, after subsequent to April, due to the high

Table 1. Prevalence of PCR-COVID-19 tests (molecular and serological) performed in Carlos Chagas Laboratory – Sabin Group laboratory in Cuiabá from April to December 2020.

PCR-COVID-19	N=23,631	%
Positive	7,649	32.37
Negative	15,982	67.63
SOROVID-19 (IgM and IgG)	N=8,884	%
Reagent	1,993	22.43
Non-reagent	6,891	77.57

Table 2. Prevalence of cases of COVID-19 disease caused by the new coronavirus (SARS-CoV-2) performed in Carlos Chagas Laboratory – Sabin Group Laboratory in Cuiabá per month, gender, and age group.

Month	Gender	Age group (year)																					
		0–9		10–19		20–29		30–39		40–49		50–59		60–69		70–79		80–89		90–100		101–109	
		(+)	(–)	(+)	(–)	(+)	(–)	(+)	(–)	(+)	(–)	(+)	(–)	(+)	(–)	(+)	(–)	(+)	(–)	(+)	(–)	(+)	(–)
		n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)
April (n=324)	Male	0	12 (3.7)	0	5 (1.5)	0	12 (3.7)	2 (0.6)	28 (8.6)	1 (0.3)	27 (8.3)	0	17 (5.2)	1 (0.3)	15 (4.6)	0	5 (1.5)	0	3 (0.9)	0	0	0	0
	Female	0	5 (1.5)	0	3 (0.9)	0	29 (9.0)	2 (0.6)	68 (21.0)	2 (0.6)	38 (11.7)	1 (0.3)	30 (9.3)	1 (0.3)	12 (3.7)	0	3 (0.9)	0	2 (0.6)	0	0	0	0
May (n=1,127)	Male	5 (0.4)	18 (1.6)	4 (0.4)	21 (1.9)	18 (1.6)	42 (3.7)	28 (2.5)	111 (9.8)	13 (1.2)	111 (9.8)	13 (1.2)	71 (6.3)	4 (0.4)	30 (2.7)	1 (0.1)	10 (0.9)	0	2 (0.2)	0	0	0	0
	Female	5 (0.4)	12 (1.1)	5 (0.4)	16 (1.4)	11 (1.0)	87 (7.7)	39 (3.5)	163 (14.5)	22 (2.0)	119 (10.6)	10 (0.9)	71 (6.3)	4 (0.4)	40 (3.5)	3 (0.3)	14 (1.2)	0	2 (0.2)	0	1 (0.1)	0	1 (0.1)
June (n=3,784)	Male	38 (1.0)	53 (1.4)	26 (0.7)	58 (1.5)	97 (2.6)	129 (3,4)	219 (5.8)	255 (6.7)	153 (4.0)	200 (5.3)	102 (2.7)	109 (2.9)	46 (1.3)	63 (1.7)	25 (0.7)	25 (0.7)	5 (0.1)	9 (0.2)	4 (0.1)	1 (0.03)	1 (0.03)	1 (0.03)
	Female	25 (0.7)	45 (1.2)	31 0.8)	76 (2.0)	172 (4.5)	220 (5.8)	281 (7.4)	413 (10.9)	161 (4.3)	275 (7.3)	130 (3.4)	133 (3.5)	59 (1.6)	64 (1.7)	23 (0.6)	22 (0.6)	13 (0.3)	15 (0.4)	1 (0.03)	3 (0.1)	1 (0.03)	2 (0.1)
July (n=5,778)	Male	46 (0.8)	108 (1.8)	49 (0.8)	92 (1.6)	150 (2.6)	223 (3.8)	267 (4.5)	373 (6.3)	207 (3.5)	241 (5.8)	109 (1.9)	171 (2.9)	57 (1.0)	117 (2.0)	28 (0.5)	52 (0.9)	7 (0.1)	10 (0.2)	1 (0.02)	1 (0.02)	0	1 (0.02)
	Female	33 (0.6)	105 (1.8)	47 (0.8)	122 (2.1)	233 (4.0)	379 (6.4)	348 (5.9)	613 (10.4)	236 (4.0)	485 (8.3)	145 (2.5)	288 (4.9)	75 (1.3)	186 (3.2)	42 (0.7)	71 (1.2)	20 (0.3)	29 (0.5)	2 (0.03)	8 (0.1)	0	1 (0.02)
August (n=2,994)	Male	19 (1.4)	62 (4.7)	23 (1.7)	60 (4.6)	72 (5.5)	124 (9.5)	100 (7.7)	236 (18.2)	101 (7.8)	184 (14.2)	67 (5.1)	98 (7.5)	35 (2.7)	48 (3.7)	8 (0.6)	37 (2.8)	3 (0.2)	9 (0.6)	1 (0.06)	6 (0.4)	0	0
	Female	10 (0.2)	54 (3.1)	29 (1.7)	64 (3.7)	77 (4.5)	178 (10.4)	147 (8.6)	304 (17.8)	118 (6.9)	256 (15.0)	63 (3.7)	144 (8.4)	42 (2.4)	105 (6.1)	16 (0.9)	55 (3.2)	3 (0.2)	30 (1.7)	1 (0.04)	5 (0.2)	0	0
September (n=2,427)	Male	12 (1.2)	59 (6.0)	11 (1.0)	39 (3.9)	59 (6.0)	105 (10.7)	99 (10.1)	171 (17.4)	62 (6.3)	135 (13.4)	46 (4.6)	64 (6.5)	22 (2.2)	43 (4.3)	15 (1.5)	22 (2.2)	2 (0.2)	10 (1.0)	1 (0.06)	3 (0.20)	0	0
	Female	12 (0.8)	37 (2.5)	21 (1.4)	59 (4.1)	78 (5.4)	193 (13.3)	110 (7.6)	319 (22.1)	80 (5.5)	197 (13.6)	46 (3.2)	113 (7.8)	27 (1.8)	86 (5.9)	14 (0.9)	34 (2.3)	9 (0.6)	10 (0.7)	0	2 (0.1)	0	0
October (n=2,040)	Male	10 (1.6)	38 (6.3)	8 (1.3)	44 (7.3)	28 (4.6)	91 (15.1)	27 (4.5)	151 (25.1)	45 (7.5)	132 (21.9)	21 (3.5)	72 (11.9)	14 (2.3)	51 (8.5)	6 (0.99)	20 (3.3)	2 (0.3)	4 (0.7)	0	0	0	0
	Female	6 (0.5)	27 (2.2)	16 (1.3)	54 (4.3)	64 (5.1)	186 (14.9)	104 (8.4)	284 (22.8)	54 (4.3)	188 (15.1)	43 (3.4)	102 (8.2)	14 (1.1)	83 (6.7)	4 (0.3)	32 (2.6)	4 (0.3)	8 (0.6)	1 (0.04)	2 (0.09)	0	0
November (n=1,626)	Male	5 (0.7)	49 (7.6)	7 (1.1)	53 (8.2)	31 (4.8)	94 (14.6)	42 (6.5)	112 (17.4)	38 (5.9)	116 (18.0)	20 (3.1)	42 (6.5)	12 (1.8)	27 (4.2)	7 (11)	9 (1.4)	0	3 (0.5)	0	0	0	0
	Female	5 (0.5)	48 (4.9)	15 (1.5)	52 (5.4)	26 (2.7)	169 (17.5)	69 (7.2)	210 (21.8)	46 (4.7)	114 (11.8)	30 (3.0)	82 (8.5)	10 (1.0)	37 (3.8)	8 (0.8)	27 (2.8)	3 (0.3)	6 (0.6)	1 (0.04)	1 (0.04)	0	0
December (n=3,531)	Male	17 (1.2)	67 (4.6)	38 (2.6)	112 (7.7)	89 (6.1)	167 (11.4)	155 (10.6)	231 (15.8)	108 (7.4)	165 (11.3)	89 (6.1)	80 (5.5)	35 (2.4)	58 (3.9)	12 (0.8)	25 (1.7)	2 (0.1)	4 (0.2)	0	2 (0.14)	0	0
	Female	18 (0.8)	65 (3.1)	50 (2.4)	114 (5.5)	137 (6.6)	254 (12.2)	218 (10.5)	383 (18.4)	138 (6.6)	276 (13.3)	68 (3.3)	168 (8.1)	32 (1.5)	80 (3.8)	18 (0.9)	37 (1.8)	8 (0.4)	8 (0.4)	0	3 (0.14)	0	0
N=23,631		266	864	380	1,044	1,342	2,682	2,257	4425	1,585	3,259	1,003	1,855	490	1145	230	500	81	164	13	38	2	6

Continue...

Table 2. Continuation.

Month	Gender	Age group (year)																					
		0–9		10–19		20–29		30–39		40–49		50–59		60–69		70–79		80–89		90–100		101–109	
		R	NR	R	NR	R	NR	R	NR	R	NR	R	NR	R	NR	R	NR	R	NR	R	NR	R	NR
		n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)
April (n=202)	Male	0	8 (4.0)	0	2 (0.5)	1 (0.5)	7 (3.5)	2 (1.0)	18 (8.9)	0	22 (10.9)	1 (0.5)	17 (8.4)	0	10 (5.0)	0	2 (1.0)	0	0	0	0	0	0
	Female	0	0	1 (1.0)	6 (3.0)	0	7 (3.5)	3 (1,5)	33 (16.3)	1 (0.5)	21 (10.4)	0	14 (6.9)	1 (0.5)	19 (9.4)	2 (1.0)	4 (2.0)	0	0	0	0	0	0
May (n=498)	Male	0	8 (1.6)	0	14 (2.8)	1 (0.2)	27 (5.4)	6 (1.2)	54 (10.9)	2 (0.4)	46 (9.3)	2 (0.4)	16 (3.2)	0	16 (3.2)	0	6 (1.2)	0	2 (0.4)	0	0	0	0
	Female	0	6 (1.2)	0	12 (2.4)	2 (0.4)	26 (5.2)	0	82 (16.5)	4 (0.8)	78 (15.7)	4 (0.8)	38 (7.7)	3 (0.6)	35 (7.7)	0	6 (1.2)	0	0	0	0	0	0
June (n=1,446)	Male	6 (0.4)	28 (1.9)	4 (0.3)	16 (1.1)	4 (0.3)	42 (2.9)	22 (1.5)	122 (8.4)	28 (1.9)	124 (8.6)	20 (1.4)	68 (4.7)	8 (0.6)	60 (4.1)	0	22 (1.5)	0	4 (0.3)	0	0	0	0
	Female	0	14 (1.0)	3 (0.3)	33 (2.3)	13 (0.9)	87 (6.0)	36 (2.5)	224 (15.5)	29 (2.0)	175 (12.1)	20 (1.4)	104 (7.2)	14 (1.0)	76 (5.3)	4 (0.3)	24 (1.7)	4 (0.3)	8 (0.6)	0	0	0	0
July (n=4,248)	Male	12 (0.3)	36 (0.8)	12 (0.3)	38 (0.9)	53 (1.2)	151 (3.6)	90 (2.3)	281 (6.6)	111 (2.6)	266 (6.3)	91 (2.1)	234 (5.5)	55 (1.3)	133 (3.1)	38 (0.9)	69 (1.6)	6 (0.1)	8 (0.2)	0	0	0	0
	Female	12 (0.3)	38 (0.9)	17 (0.4)	63 (1.5)	75 (1.8)	287 (6.8)	138 (3.2)	446 (10.5)	160 (3.8)	399 (9.4)	121 (2.8)	325 (7.7)	102 (2.4)	216 (5.1)	38 (0.9)	58 (1.4)	10 (0.2)	32 (0.8)	0	0	0	0
August (n=1,247)	Male	10 (1.9)	12 (2.2)	8 (1.5)	14 (2.6)	16 (3.0)	35 (6.6)	30 (5.6)	79 (14.9)	36 (6.8)	69 (12.9)	38 (7.1)	64 (12.0)	27 (5.1)	43 (8.1)	13 (2.4)	7 (1.3)	1 (0.2)	7 (1.3)	1 (0.2)	3 (0.6)	0	0
	Female	8 (1,11)	16 (2.7)	10 (1,4)	14 (1.9)	13 (1.8)	52 (7.3)	41 (5.7)	111 (15.5)	40 (5.6)	116 (16.2)	37 (5.2)	86 (12.0)	31 (4.3)	75 (10.5)	14 (1.9)	19 (2.6)	5 (0.7)	19 (2.6)	1 (0.1)	3 0.4)	0	0
September (n=414)	Male	3 (1,5)	6 (3.1)	2 (1,0)	5 (2.6)	3 (1.5)	11 (5.6)	6 (3.1)	29 (14.9)	10 (5.1)	32 (16.4)	10 (5.1)	30 (15.4)	6 (3.1)	23 (11,8)	0	6 (3.1)	3 (1,5)	6 (3.1)	0	2 (2.0)	0	0
	Female	0	6 (2.7)	2 (0,9)	7 (3.2)	5 (2.3)	19 (8.7)	7 (3.2)	34 (15.5)	9 (4.1)	36 (16.4)	7 (3.2)	29 (13.2)	8 (3.6)	21 (9.6)	4 (1.8)	6 (2.7)	2 (0.9)	6 (2.7)	0	1 (0.5)	0	0
October (n=331)	Male	1 (0.7)	2 (1.4)	0	5 (3.5)	2 (1.4)	4 (2.8)	7 (4.9)	17 (11.9)	5 (3.5)	31 (21.8)	5 (3.5)	23 (16.1)	5 (3.5)	23 (16.1)	0	9 (6.3)	0	2 (1.4)	0	0	0	0
	Female	1 (0.5)	1 (0.5)	1 (0,5)	3 (1.6)	4 (2.1)	14 (7.4)	4 (2.1)	32 (16.9)	5 (2.6)	36 (19.0)	5 (2.6)	34 (18.0)	5 (2.6)	21 (11.1)	2 (1.0)	15 (7.9)	0	5 (2.6)	0	1 (0.5)	0	0
November (n=184)	Male	0	4 (5.7)	1 (1,4)	2 (2.8)	1 (1.4)	2 (2,8)	1 (1.4)	9 (12.8)	1 (1.4)	18 (25.7)	1 (1.4)	11 (15.7)	3 (4.3)	12 (17.1)	0	4 (5.7)	0	0	0	0	0	0
	Female	0	2 (1.7)	0	5 (4.4)	0	19 (16.7)	1 (0.9)	20 (17.5)	5 (4.4)	19 (16.7)	3 (2.6)	15 (13.1)	6 (5.2)	15 (13.1)	0	1 (0.9)	2 (1.8)	0	0	1 (0.9)	0	0
December (n=379)	Male	1 (0.5)	5 (2.6)	0	8 (4.1)	2 (1.0)	15 (7.6)	4 (2.0)	24 (12.2)	6 (3.0)	27 (13.8)	5 (2.5)	27 (13.8)	4 (2.0)	17 (8.7)	0	10 (5.1)	0	2 (1.0)	0	0	0	0
	Female	0	4 (2.2)	0	8 (4.4)	2 (1.1)	18 (9.8)	5 (2.7)	43 (23.5)	12 (6.5)	41 (22.4)	7 (3.8)	28 (15.3)	6 (3.3)	26 (14.2)	2 (1.1)	12 (6.5)	1 (0.5)	6 (3.3)	0	1 (0.55)	0	0
N=8,884		54	196	61	255	197	823	403	1,658	464	1,556	377	1,163	284	841	117	280	34	107	2	12	0	0

R: reagent; NR: no reagent.

rate of transmission and community circulation of SARS-CoV-2. There was a predominance of 5,258 (59.18%) tests in women, reaching the majority between the age group of 20 and 59 years. The reagent results were registered with a peak in July (n=1,169; 57.16%), entitled as the first wave of infection in Brazil, totaling 4.21 examinations performed in that month for IgM and IgG, undetected (n=6,981) (Table 2).

The cases confirmed by PCR-COVID-19 data between April and December 2020, and data series represent the mean and respective 95%CI of PCR-COVID-19 cases for each age group, and data series represent the positive numbers of SOROVID-19 (IgG/IgM) between April and December 2020 in Carlos Chagas Laboratory – Sabin Group in Cuiabá (Figure 1).

No temporal trend was observed with either SOROVID (p=0.561) or PCR (p=0.289) (Figure 1).

DISCUSSION

According to our results, there was a first wave of COVID-19 in Cuiabá in July and later a severe decrease in molecular and serological positivity, with the beginning of a second wave, starting in December 2020, which in fact was observed in Brazil from January 2021. Our data are in accordance with the high rate of transmission and community circulation of SARS-CoV-2 in Cuiabá during the analyzed period (Figure 1A–D), characterizing the month of July as the epicenter of COVID-19 the central west region of Brazil[14,15].

The results of the 23,631 tests performed for SARS-CoV-2 in the period from April to December detected 32.37% of the positive tests by the molecular test (RT-PCR) (Table 1) and 47.51% of the serological tests by the SOROVID-19 test (IgG/IgM); of the positive tests, 1,169 (57.16%) for SARS-CoV-2

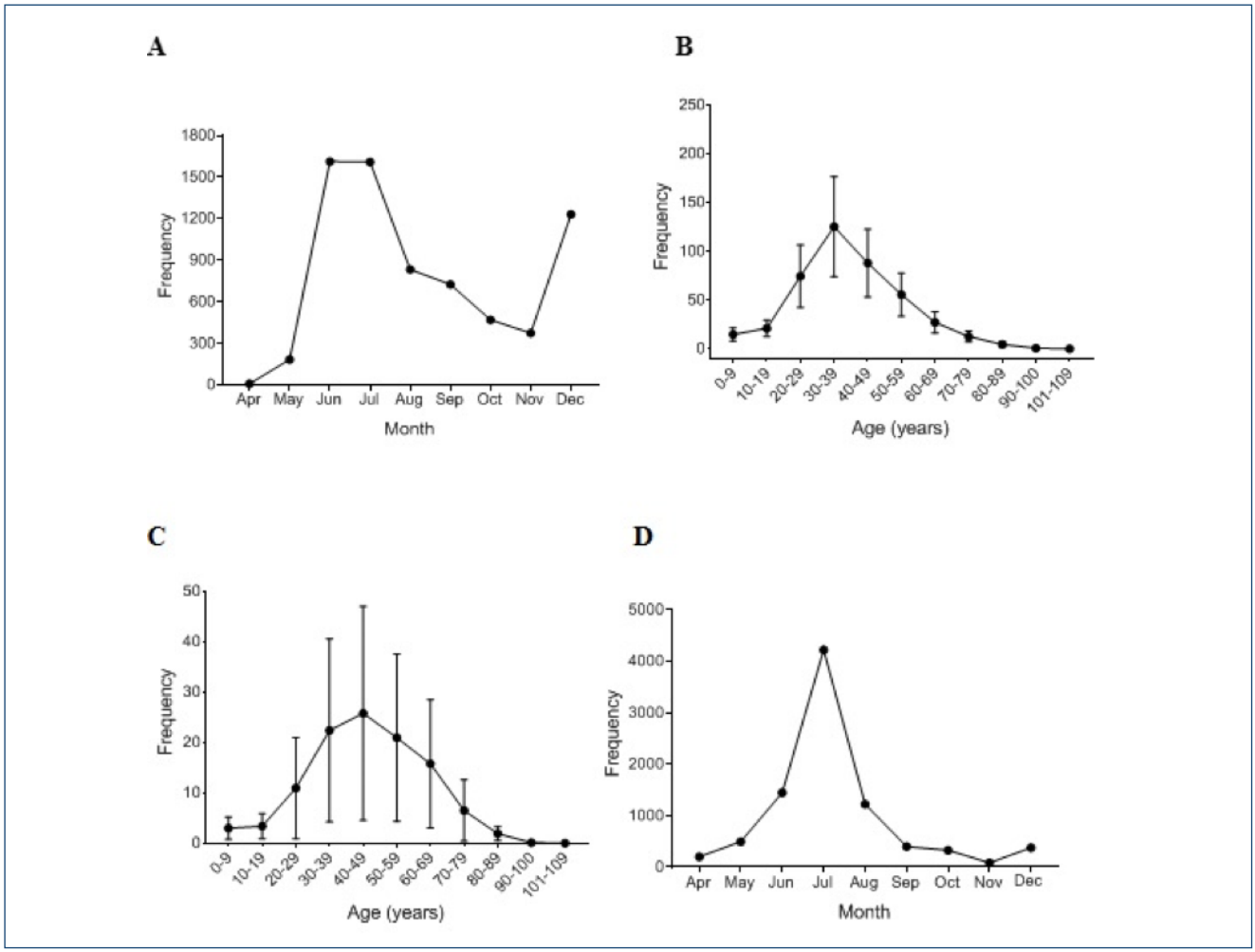

Figure 1. **(A)** Cases confirmed by PCR-COVID-19 between April and December 2020 in Carlos Chagas Laboratory – Sabin Group in Cuiabá. p=0.289 (time trend, Cuzik test). **(B)** Frequency of COVID-19 cases detected by RT-PCR in Carlos Chagas Laboratory – Sabin Group in Cuiabá, from April to December 2020. Data series represent the mean and respective 95%CI. **(C)** Data series represent the mean and respective 95%CI of PCR-COVID-19 cases for each age group in Carlos Chagas Laboratory – Sabin Group in Cuiabá. p=0.561 (time trend, Cuzik test). **(D)** Data series represent the positive numbers of SOROVID-19 (IgG/IgM) between April and December 2020 in Carlos Chagas Laboratory – Sabin Group in Cuiabá.

in July, with women in the age group of 20–59 years being predominant (Figure 1).

Regarding gender, the incidence of SARS-CoV-2 was more positive in women, similar to the findings in another study conducted in Mato Grosso and Rio de Janeiro[10] which also reported the majority of cases in women (51.4%), whereas in men it was 47.7%. In contrast to our study in Wuhan Province, China[19], the prevalence was higher in men (56%) with mortality 56.5 and 38.0% of female deaths[10].

According to data from the State Department of Health, Cuiabá, Várzea Grande, and 13 other cities in Mato Grosso are classified as "very high" risk for the new coronavirus. This risk classification points to cities with more than 150 active cases on that date, such as Sorriso with 24.81%, Barra do Garças with 19.92%, and Paranatinga with 14.83%, the other cities in the State are between 2 and 11%. In Cuiabá, there are 13,958 confirmed cases with 636 deaths and the city Várzea Grande with a high mortality rate, as there are 5,234 confirmed cases with 337 deaths and a 7% lethality rate, confirming that the Baixada Cuiabana has a lethality rate above the national average[14,15].

According to the Epidemiological Bulletin of the State of Mato Grosso, the profile of patients with COVID-19 is predominantly women (52%), as well as the prevalence of deaths is also higher in women (59.3%)[14,15]. However, this distribution differs between the states, as in Maranhão where the death due to COVID-19 was predominant in men (62%)[16]. It is believed that women seek health services more frequently than men, and there may be underreporting of cases in the male population, as, historically, men seek health services less, which can lead to the worsening of the disease, late treatment, and evolution to death.

Regarding the age group, there was a predominance of cases of patients between 20 and 59 years for both tests, i.e., molecular and serological, for the detection of SARS-CoV-2. Those findings are similar to the ones found in a study carried out in Maranhão (28.4%)[16] and in Wenzhou (China), which presented 58.9% of cases in the same age group[17]. Likewise, individuals aged 30–59 years were more prevalent among the cases studied in Rio de Janeiro[10]. It is worth emphasizing the need to endorse nonpharmacological measures, in order to reduce the number of people with the disease in the same age group, which characterizes the economically active population and reinforces the adoption of assertive socioeconomic measures and preventive measures with the epidemiological surveillance of each citizen to decrease the transmissibility of SARS-CoV-2[19].

The serological methods have public health value for monitoring and responding to the COVID-19 pandemic and clinical utility in providing care for patients. Our results showed that the detection of antibodies was mostly in July due to the effect of the first wave COVID-19 in Brazil and characterized of the immunological window period. Moreover, the serological tests may be negative in asymptomatic patients or those who did not report the onset period of symptoms for SARS-CoV-2 IgM/IgG positivity as recommended by the Ministry of Health (10–12 days)[12].

Among the limitations, despite the secondary data of this study being collected in a locally and nationally known laboratory, the samples are representative and descriptive only from the city of Cuiabá and the region roundabout. This in fact precludes a statewide coverage of the epidemiology of COVID-19, as well as the possibility that the population may have performed tests for SARS-CoV-2 in other laboratories available in the capital. However, it is one of the first studies describing the cases of COVID-19 and the type of approach carried out in Cuiabá which directly contributes to decision-making by requiring notification to the surveillance and health control bodies.

CONCLUSION

Therefore, we conclude that the prevalence of COVID-19 in Cuiabá – MT was higher in women aged 20–59 years and the number of confirmed cases was higher from June to July 2020. The amount of detection of examinations by RT-PCR and reagents for SOROVID (IgM and IgG) monthly increased, having its peak in July 2020, which in fact reflects the high transmissibility rate and first wave of infection of SARS-CoV-2 in Cuiabá with a public health emergency.

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AUTHOR'S CONTRIBUTIONS

CCP: Designed the study, wrote the manuscript, coordinated the study. **WS-B:** Performed the experiments, wrote the manuscript. **JPCP:** Performed the experiments. **CAVDLP:** Designed the study. **KA:** Language correction, manuscript writing and drafting. **CJFF:** Statistical analysis (Cuzik test was used to analyse the time trend across the month). **RGO:** Designed the study, wrote the manuscript, coordinated the study.

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ORIGINAL ARTICLE

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COVID-19 infection rates among transportation and metal workers

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SUMMARY

OBJECTIVE: The aim of this study was to compare the workers in the metal and transportation sectors in terms of COVID-19 infection frequency and to examine and establish links between infection frequency and the workplace working conditions.

METHODS: A survey was prepared and conducted with a questionnaire prepared on the Google Form platform consisting of questions about the pandemic among the members of the All Transport Workers' and The United Metalworkers' Union in Turkey.

RESULTS: The number of workers diagnosed with COVID-19 was 5.8% in the transportation sector and 2.8% in the metal sector, with a significant difference (p=0.036). The percentage of workers diagnosed with COVID-19 who worked at a physical proximity less than 2 m in the transportation sector was higher than those who worked in the metal sector (p=0.014). The proportion of those who stated that there were COVID-19 patients among their colleagues and working at a physical proximity less than 2 m at the workplace was 18.2% in the transportation and 10.6% in the metal sector, with a significant difference (p=0.003), those who took time off from work was 74%, but 28.5% successively (p<0.001). The share of those who thought that the protective equipment and/or measures were not sufficient during the pandemic was 41.9% in the transportation and 17.7% in the metal sector (p<0.001).

CONCLUSION: The results emphasized that the characteristics of jobs, physical proximity during job hours, the use of protective equipment, and size of the workplaces should be considered as reasons for different infection risks in different sectors.

KEYWORDS: COVID-19. Infection. Metal workers. Transportation.

INTRODUCTION

The coronavirus disease 2019 (COVID-19) infection, which poses a high level of risk for some occupational groups such as the health care sector, also affects workers in other industrial branches where individuals have close contact with each other and share crowded and unprotected environments such as transportation services, lockers, restrooms, and cafeterias. Therefore, travel has been restricted to prevent the spread of COVID-19 infection in the world, and governments have taken measures such as social distancing, encouraging staying at home, education, and working from home[1].

Health care, security, retail, and cargo sectors include occupational groups that have provided uninterrupted service even during the pandemic. A study including six Asian countries showed that 15% of 690 COVID-19 infection cases were work related. Health care workers (22%), drivers (18%), cleaners (9%), and security guards (7%) were the most frequently affected work-related disease groups[2]. Of the 25 local spread cases reported from Singapore, 17 were work related, including tourism workers, sales and accommodation workers, transportation, and security personnel[3]. A literature review by Souza et al. showed that the highest COVID-19 prevalences were observed among farmers (20.76%) and retired persons (19.77%). Working in the service industry and health sector was seen in 7.19 and 4.34% of patients with COVID-19, respectively[4].

Pouliakas and Branka used data from the Cedefop European skills and jobs survey and created a COVID-19 social distancing risk index (COV19R) based on skills descriptors that categorize jobs by their level of physical proximity to others and their digital intensity. Their analysis showed a lowest COV19R value among metal and machinery workers compared with other workers[5].

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The aim of our study was to compare the workers working in the automotive supply industry and cargo workers in terms of the frequency of COVID-19 infection and to examine the links between the workplace, working conditions, and the frequency of COVID-19 infection.

METHODS

This research was conducted by conducting a survey created on the Google Form platform consisting of questions about the COVID-19 epidemic to the workers through unions and some social networks. In this context, the members of the United Metalworkers' Union organized in the metal sector, especially the automotive supply industry, and the members of the TUMTIS union organized in the transportation sector, especially cargo companies were determined as the target population. A total of 866 surveys were included in the evaluation. The survey was administered between June 2 and June 17, 2020. The surveys were analyzed using SPSS software package (version 24). The Pearson's chi-square test was applied to analyze whether the correlation between two variables was significant in categorical comparisons. The Fisher's exact chi-square test was applied if one or more of the cells had a value of 5 or less. In the case of p-value <0.05, it was concluded that there was a significant difference between the two categories in terms of relevant variables.

RESULTS

Demographic characteristics of the sample population and chronic diseases, smoking, and alcohol consumption among them

The mean age of the metal workers who participated in the study was 36 years (SD: 8), and the mean age of the cargo workers was 38 years (SD: 8).

There was a significant difference between the two sectors in terms of gender distribution. The proportion of women was 14.6% in the transportation sector and 6.5% in the metal sector (p<0.001).

Moreover, the proportion of workers with chronic diseases was close to each other in the two sectors, with 14.7% in the transportation sector and 12.1% in the metal sector. The difference was not statistically significant.

There was a significant difference between the two sectors in terms of smoking, with the proportion of smokers 58.6% in the transportation sector and 50.1% in the metal sector (p=0.041).

COVID-19 infection characteristics of workers in the transportation and metal sectors

The proportion of workers diagnosed with COVID-19 was 5.8% in the transportation sector and 2.8% in the metal sector, showing a significant difference between the two sectors in terms of the diagnosis of COVID-19 (p=0.036).

Out of 356 workers in the transportation sector, 20 workers stated that they were diagnosed with COVID-19; and out of 433 workers in the metal sector, 12 workers stated that they have been diagnosed with COVID-19. Among the 20 workers with COVID-19 in the transportation sector, 11 out of 16, who stated their sectors, were working in cargo and courier-related jobs.

Table 1 shows the comparison of workers diagnosed with COVID-19 by sectors. Accordingly, there was no significant difference between the two sectors in terms of pharyngeal/nasal/swab testing. A similar case applies to the result of a positive test. There was no significant difference between the workers working in the two sectors in terms of the symptoms of the disease.

Table 1 shows whether there are other individuals with COVID-19 patients at home or at work among the workers diagnosed with COVID-19. There was no significant difference in those diagnosed with COVID-19 according to whether there is another household member diagnosed with COVID-19. In contrast, those who have COVID-19 cases among their colleagues who work at a physical proximity less than 2 m showed a statistically significant difference in terms of the two sectors (75% in the transportation sector vs. 20% in the metal sector, p=0.014).

The question "Is/was there anyone in the household with COVID-19?" was asked to all workers who responded to the survey, and no significant difference was found between the transportation and metal sectors. In contrast, there was a statistically significant difference between the workers who responded to the question "Is/was there any COVID-19 patients among your other colleagues who worked at a physical proximity less than 2 m to you at your workplace?" by their sectors. While the proportion of those who responded yes to this question was 18.2% in the transportation sector, it was 10.6% in the metal sector (p=0.003).

Moreover, there were significant differences in terms of taking time off from work in this period by sectors. Short-time working has been implemented at the workplaces, activities of which decreased immediately after the effects of the crisis in the metal sector. In contrast, the transportation sector became one of the critical sectors and continued to work. In fact, the proportion of those who stated that they took off from work in this period was 74% in the metal sector and 28.5% in the transportation sector (p<0.001). Furthermore, the proportion of workers at

Table 1. Positive test results and disease symptoms by sectors.

		Comparison of metal and transportation sectors				
		Transportation		Metal		Fisher's exact test
		N	N%	N	N%	Exact significance (two-sided)
Have you had pharyngeal/nasal/swab testing with the diagnosis of COVID-19?	No	4	20.0	3	25.0	1.000
	Yes	16	80.0	9	75.0	
Is the test positive or negative?	Negative	5	31.3	3	30.0	1.000
	Positive	11	68.8	7	70.0	
Cough	No	9	56.2	6	66.7	0.691
	Yes	7	43.8	3	33.3	
Shortness of breath	No	14	73.7	10	83.3	0.676
	Yes	5	26.3	2	16.7	
High fever	No	12	63.2	11	91.7	0.108
	Yes	7	36.8	1	8.3	
Arthralgia	No	14	87.5	8	88.9	1.000
	Yes	2	12.5	1	11.1	
Loss of taste and smell	No	14	87.5	8	88.9	1.000
	Yes	2	12.5	1	11.1	
Fatigue	No	15	93.8	9	100.0	1.000
	Yes	1	6.3	0	0.0	
No complaint	No	13	81.3	6	66.7	0.630
	Yes	3	18.8	3	33.3	
Unresponded	No	16	84.2	9	75.0	0.653
	Yes	3	15.8	3	25.0	
Was there any other COVID-19 patient at home at the time of diagnosis?	No	15	93.8	6	66.7	0.116
	Yes	1	6.3	3	33.3	
Are/were there any COVID-19 patients among your colleagues working away from you at your workplace?	Yes	11	78.6	5	55.6	0.363
	No	3	21.4	4	44.4	
Were there any COVID-19 patients among your other colleagues who worked at a physical proximity less than 2 m to you in your workplace?	Yes	12	75.0	2	20.0	0.014
	No	4	25.0	8	80.0	

the time of the survey was 91.9% in the transportation sector and 70.9% in the metal sector (p<0.001). The proportion of those who stated that they benefited from short-time work was 6.1% in the transportation sector and 45.2% in the metal sector.

Regarding all of the workers from two sectors diagnosed with COVID-19, 36.36% had COVID-19 patients at home. In contrast, this rate was 2.6% in those who did not have the disease (p<0.001).

The proportion of individuals diagnosed with COVID-19 who worked with another worker with COVID-19 diagnosis at a physical proximity less than 2 m at the workplace was 53.9%, with a significant result (p<0.001), while it was found to be 12.8% among those who were not diagnosed with COVID-19.

Within the scope of the study, it was questioned whether the measures taken against COVID-19 at the workplaces made a significant sectoral difference. Accordingly, there was no significant difference between the two sectors in terms of the question of whether employers took measures (p=0.101). The proportion of those who thought that the protective equipment and/or measures were not sufficient during the pandemic was

41.9% in the transportation sector and 17.7% in the metal sector. The difference was statistically significant (p<0.001).

There were significant differences between the transportation and the metal sectors in terms of providing protective equipment and taking measures, except for masks (Table 2). The proportion of workers stating that other than protective equipment, measures such as "marking for social distance," "reducing the number of workers in the personnel shuttle," and "adjusting the seating in the cafeteria" were taken at the workplace was higher than those in the metal sector. In contrast, gloves and face shields were provided to the workers in the transport sector at a higher rate.

DISCUSSION

Our study comparing the frequency of COVID-19 infection, workplace conditions, and disease characteristics of patients among metal workers and cargo and transportation workers is the first to compare the moderate-low risk group of metal sector and the high-risk group of cargo and transportation sector in terms of COVID-19 infection. In the study, the rate of infection in cargo and transportation sector workers (5.8%) was twice as high as in the metal sector (2.8%), especially in the automotive industry. It was observed that workers in the transportation and cargo sector reported a higher proportion of patients with COVID-19 among their colleagues working at a close vicinity than those in the metal sector (18.3% vs. 10.6%). The proportion of those who thought that the protective equipment and/or measures taken were not sufficient during the pandemic was significantly higher in the transportation and cargo sector than in the metal sector (41.9 vs. 17.7%).

Of more than 130,000 workers working in meat and poultry processing facilities in the United States (in 19 states), the proportion of those who were infected with the disease during the pandemic was 3%, with a mortality rate of 0.04%. Conditions that pose risks for workers in the sector during the pandemic were listed. It was observed that a safe distance could not be provided between the workers during breaks and working hours, with problems in wearing masks and adhering to

Table 2. Provision of protective equipment among metal and transportation workers.

		Comparison of metal and transportation sectors				
		Transportation		Metal		Chi-square tests
		N	N%	N	N%	Asymptotic significance (two-sided)
Did the employer take any measure against COVID-19 (including providing protective equipment to you) during this period?	No	37	10.90	32	7.50	0.101
	Yes	303	89.10	396	92.50	
Do you think that the protective equipment and/or measures taken during the pandemic were sufficient?	No	143	41.90	75	17.70	<0.001
	Partially	67	19.60	163	38.40	
	Yes	131	38.40	186	43.90	
Mask	No	16	4.60	28	6.50	0.268
	Yes	330	95.40	405	93.50	
Gloves	No	66	19.10	184	42.50	<0.001
	Yes	280	80.90	249	57.50	
Face shield	No	168	48.60	286	66.10	<0.001
	Yes	178	51.40	147	33.90	
Apron	No	333	96.20	397	91.70	0.009
	Yes	13	3.80	36	8.30	
Disinfectant	No	81	23.40	64	14.80	0.002
	Yes	265	76.60	369	85.20	
Marking for social distancing	No	217	62.70	151	34.90	<0.001
	Yes	129	37.30	282	65.10	
Reducing the number of workers in the personnel shuttle	No	191	55.20	101	23.30	<0.001
	Yes	155	44.80	332	76.70	
Arranging the seating in the cafeteria	No	251	72.50	78	18.00	<0.001
	Yes	95	27.50	355	82.0	

disinfection rules. The measures to be taken were reported as ensuring the safe distance among the workers, increasing the number of shuttles, and increasing education[6]. In our study, the level of measures including the use of disinfectants (85.2 vs. 76.6%), marking for social distance (65.1 vs. 37.3%), reducing the number of workers in the shuttle (76.7 vs. 44.8%), and adjusting the seating in the cafeteria (82 vs. 27.5%) was significantly higher in the metal sector compared to the cargo and transportation companies. The rate of reporting the provision of an adequate number of masks was between 93 and 95% in both sectors. The provision of gloves (80.9 vs. 57.5%) and face shields (51.4 vs. 33.9%) was found to be higher in the cargo and transportation sector than in the metal sector. Unlike the study conducted in the United States, our study revealed the measures taken in different areas of the workplace in more detail since it used the data obtained from the workers via surveys. Our study was conducted 4 months after the onset of the pandemic and shows that the measures were taken more effectively in the metal sector. According to the results of the study, whether or not to take off from work was one of the issues that produced a significant difference for the two sectors. While the metal sector is a business line where short-time work is implemented intensively due to the decrease in production, it is not possible to mention a decrease in the activities of the transportation sector. This may be one of the sources of the difference between the two sectors.

It is seen that there is a remarkable difference in terms of workplace scale size and unionization rates for both sectors. The metal sector has a unionization rate of 3.6 points (17.4%), which is above the Turkish average of 13.8%. In contrast, the rate of organization in the transportation sector is 10.5%, which is below the Turkish average[7,8].

It can be speculated that variables such as sectoral differences, the nature of the work, the level of institutionalization of the workplaces, and the capacity of workers to act in an organized manner affect the spread rate of COVID-19 at workplaces. However, it is obvious that workplaces do provide suitable environments for the spread of the virus no matter how effective are the measures taken at the workplaces.

CONCLUSION

This study attempted to address the risks faced by workers in two sectors with different qualitative characteristics in terms of COVID-19 in the early stage of the pandemic through the measures taken in the workplace and the nature of the work. The results of this study will make a significant contribution to the literature and future studies in terms of the correlation of the epidemic with the working environment.

AUTHORS' CONTRIBUTIONS

FSÖ: Conceptualization, Data curation, Formal Analysis, Writing – original draft. **NG:** Conceptualization, Data curation. **GEG:** Formal Analysis, Writing – review & editing. **PA:** Conceptualization, Writing – review & editing.

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ORIGINAL ARTICLE

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Correlation between the range of motion of the tibiotarsal joint and blood circulation in the lower limbs in diabetic individuals

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SUMMARY

OBJECTIVE: The aim of this study was to evaluate the relationship between the range of motion and lower-limb hemodynamic indices in the tibiotarsal joint of individuals with diabetic neuropathy.

METHODS: Twenty volunteers of both sexes, with a mean age of 61.45±7.05 years, were diagnosed with type 2 diabetes mellitus and diabetic peripheral neuropathy. Arterial blood flow was assessed using Doppler ultrasound, and the variables such as average velocity, pulsatility index, and resistivity index were also evaluated. A range of dorsiflexion and plantar flexion joint movements were assessed using digital goniometry before and after exercise. Data distribution was assessed using the Shapiro-Wilk test, followed by Pearson's correlation for normal data and Spearman's correlation for non-normal data, in order to verify the association between variables.

RESULTS: A moderate correlation was found between dorsiflexion and pulse rate on two occasions before (rs=0.497) and after initial evaluation (rs=0.511). A low correlation was found between plantar flexion and mean velocity (rs=-0.357), pulsatility index (rs=0.439), and resistivity index (rs=0.328); dorsiflexion and mean velocity (rs=0.374), pulse rate (rs=0.332), and resistance index (rs=0.327) before evaluation, and peak (rs=0.346) was observed after the evaluation of blood circulation.

CONCLUSION: There is a correlation between the range of motion of the tibiotarsal joint and the blood circulation of diabetics, ranging from moderate to poor for the different variables evaluated.

KEYWORDS: Physical therapy modalities. Diabetes mellitus. Ankle joint.

INTRODUCTION

Diabetes is a chronic disease whose complication causes damage to health and is the primary cause of cardiovascular disease and death today[1]. The number of people with diabetes mellitus worldwide is estimated to increase by 54%, from 285 million in 2010 to 439 million by 2030[2]. The high incidence can be explained by a rapid change in diet, coupled with sedentary habits, which increases the number of cases with chronic disease, highlighting obesity and type 2 diabetes[3].

Peripheral neuropathy, which affects the integrity of peripheral nerves, is one of the main complications of diabetes and is caused by high blood glucose levels that promote the accumulation of tissue metabolism end products[4]. This process leads to a loss of protective sensitivity and musculoskeletal function of the lower limbs[5].

Joint mobility and muscle function are impaired in diabetes due to the nonenzymatic collagen glycosylation process that damages the joint structure, ligaments, and tendons, thus compromising the elasticity and tensile strength of these structures[6]. The talofibular joint is the most affected joint due to the biomechanical changes related to multiple factors of neural and mechanical deficit which interfere with the function of intrinsic foot muscles, causing changes in balance, plantar pressure, and articular mobility with influence on gait, and on motor function, altogether in patients with neuropathic diabetes[7].

Peripheral arterial disease in diabetes is manifested by atherosclerosis, resulting from the harmful effect of hyperglycemia on the vascular endothelium, where atheromatous plaques cause limb artery blockage, especially in the lower limbs[8]. It is a disease where few patients are symptomatic. Of those with symptoms, intermittent discomfort is reported due to leg pain caused by physical effort, and this is relieved by resting[9].

Factors that directly influence the function of movement, and thus hinder the daily life of patients, are the reduced range of motion and lower-limb blood circulation[10].

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Given the above, the need to investigate the influence of joint mobility on the lower-limb blood circulation of individuals affected by diabetes, who developed peripheral neuropathy, is justified in order to substantiate clinical practice. This study aimed to evaluate the correlation between the range of motion of the tibiotarsal joint and lower-limb blood circulation in individuals with diabetic neuropathy.

METHODS

Ethical aspects

This study was conducted at the Ribeirão Preto Medical School (FMRP-USP) from February 2018 to March 2019. It was approved by the Research Ethics Committee of the Ribeirão Preto Clinical Hospital, Ribeirão Preto Medical School (protocol 808/2017). It was conducted by the National Health Council Resolution 466/12, and all patients agreed and signed a free and informed consent form.

Sample size

The sample size was calculated based on the study conducted by Marrón-Gómez et al.[11], considering the range of motion as an outcome variable, with a statistical power of 80% and significance level (alpha) of 5%, revealing n=18. Based on this, and predicting possible sample losses, a total of 20 patients were selected for this study.

Patients with cutaneous lesions or lower-limb fractures in the past 6 months, plantar malformations, severe postural changes, and/or a real difference in lower-limb length were excluded from the study.

Evaluation procedures

Evaluations were performed in the morning, on a single day, to limit the effects of daily hormonal variations[12]. The measurements were taken before and immediately after the exercise. All participants underwent standard anamnesis. The characterization of the sample in relation to the risk of peripheral arterial disease was quantified by calculating the ankle/brachial index (ABI) using the SONARA/Tek® Doppler ultrasound (Nicolet Vascular, Madison, USA). The measurements were performed in the supine position after 5 min of rest. The values considered were between 0.90 and 1.40, with indices >1.40, representing an increase in arterial resistance, and indices £0.90, demonstrating the presence of peripheral arterial disease[13].

For the range of motion analysis of the ankle joint, a digital goniometer (Richmeters®) was used in the supine position with slight knee flexion and the feet at 90º. Dorsiflexion and plantar flexion movements of each foot were analyzed, and the average of three repetitions was recorded[14]. For thermographic analysis of vasomotor aspects (points), the FLIR® model T300 W/25 térmica thermal camera (FLIR® Systems, Wilsonville, OR, USA), with a sensitivity of 0.1°C and focal plane matrix of 320/240, was used. Skin temperature (solar) was assessed after 15 min of acclimatization at environmentally controlled temperature of 22±2°C[15], in a place illuminated by fluorescent lamps, without the presence of heat-generating electrical equipment and light. To evaluate the blood flow, the SONARA/Tek® Doppler ultrasound (Nicolet Vascular, Madison, USA), calibrated with an 8 MHz probe, was used at 45º to the blood vessel and on skin greased with water-soluble gel[16]. The measurements were performed in the supine position with the lower limbs in extension, and the blood vessels analyzed were the posterior tibial artery and the dorsal artery of the feet.

Neurological impairment was assessed using the diabetic distal polyneuropathy diagnostic scale and was translated into Portuguese and tested for reliability by Moreira et al.[17]. This is a tool used for assessing neurological symptoms such as muscle weakness, sensory disturbances, and autonomic symptoms. The scale has a score ranging from 1 to 10, with 1 indicating no neuropathic symptoms, 3–4 indicating mild neuropathic symptoms, 5–6 indicating moderate neuropathic symptoms, and 7–10 indicating severe symptoms. To evaluate a circulatory response after mobilization, an exercise protocol was used in which the patient in supine position with supported lower limbs performed plantar flexion and dorsiflexion (3 sets of 15 repetitions), and circular movements of the ankle (3 sets of 15 repetitions)[18].

Statistical analysis

The Shapiro-Wilk test was used to verify the distribution of the data. The range of motion and the surface temperature of the skin had a normal distribution, which was analyzed using Pearson's correlation coefficient. The association between blood flow and range of motion had a non-normal distribution, which was analyzed using the Spearman's correlation coefficient.

To interpret the magnitude of the correlations, we used the following classification established by Munro[19]: low, 0.26–0.49; moderate, 0.50–0.69; high, 0.70–0.89; and very high, 0.90–1.00.

RESULTS

Neurological symptoms in the lower limbs, such as sensory disturbances and autonomic symptoms, were determined using the Diabetic Distal Polyneuropathy Diagnostic Scale.

Severe symptoms and peripheral obstructive arterial disease were predominant. The ABI values of all volunteers remained between 0.90 and 1.40, which are considered normal.

The sample characterization of 20 patients was done on average, followed by the standard deviation of the patients' age of 61.45±7.05 years, body mass index of 32.47±5.70 kg/cm^2, and time of diabetes diagnosis of 11.6±6.64 years.

Correlations were low (from 0.26 to 0.49) between right plantar flexion and temperatures of the right lower limb both before and immediately after dynamic assessment, and also between left plantar flexion and temperatures of the left-leg regions at the post-immediate moment (Table 1).

The correlations were low (from 0.26 to 0.49) between joint movement (plantar flexion and dorsiflexion) and the values of mean velocity, peak, pulsatility index, and resistivity index of arterial blood circulation of both arteries, i.e., posterior tibial artery and dorsal artery of the right leg, were analyzed at the pre- and post-immediate moments (Table 2).

Table 1. Results of correlation between range of motion and surface temperature at the pre- (PRE) and post-immediate (POST) moments of dynamic evaluation.

Region	PRE				POST			
	R PF (r-value)	p	R DF (r-value)	p	R PF r-value)	p	R DF (r-value)	p
Front	0.302	0.196	-0.224	0.342	0.213	0.367	-0.08	0.736
Side	0.283	0.227	-0.153	0.520	0.281	0.230	-0.03	0.902
Medial	0.252	0.283	-0.223	0.344	0.268	0.253	-0.074	0.757
Back	0.296	0.204	-0.126	0.597	0.331	0.154	-0.145	0.541
Region	**L PF (r-value)**	**p**	**L DF (r-value)**	**p**	**L PF (r-value)**	**p**	**L DF (r-value)**	**p**
Front	0.113	0.635	-0.318	0.172	0.283	0.227	-0.100	0.676
Side	0.132	0.579	-0.181	0.444	0.278	0.235	-0.082	0.730
Medial	0.179	0.449	-0.311	0.181	0.249	0.290	-0.110	0.643
Back	0.141	0.553	-0.294	0.208	0.148	0.534	-0.255	0.279

Values presented in correlation index (r-value) and p-value. Right plantar flexion (R PF), right dorsiflexion (R DF), left plantar flexion (L PF), left dorsiflexion (L DF), front region leg (Front), side region leg (Side), medial region leg (Medial), back region leg (Back). Positive value: >0; negative value: <0.

Table 2. Results of correlation between range of motion and blood circulation of the right lower limb at the pre- (PRE) and post-immediate (POST) moments.

Right tibial	PRE				POST			
	R PF (r-value)	p	R DF (r-value)	p	R PF r-value)	p	R DF (r-value)	p
MEAN	-0.203	0.390	-0.390	0.089	-0.334	0.150	-0.129	0.354
PEAK	-0.049	0.838	-0.150	0.528	-0.287	0.220	-0.130	0.586
PI	0.283	0.227	0.096	0.688	0.032	0.892	0.394	0.086
RI	0.015	0.950	-0.006	0.980	0.016	0.946	-0.110	0.645
Right dorsal	**R PF (r-value)**	**p**	**R DF (r-value)**	**p**	**R PF r-value)**	**p**	**R DF (r-value)**	**p**
MEAN	-0.357	0.122	0.374	0.104	0.102	0.670	-0.134	0.573
PEAK	-0.234	0.321	0.162	0.495	0.326	0.160	-0.005	0.833
PI	0.020	0.935	-0.335	0.148	0.439	0.053	0.014	0.955
RI	-0.091	0.703	0.164	0.489	0.328	0.158	-0.017	0.943

Values presented in correlation index (r-value) and p-value. Right plantar flexion (R PF), right dorsiflexion (R DF), right posterior tibial artery (RIGHT TIBIAL), right dorsal artery (RIGHT DORSAL), mean arterial flow velocity (MEAN), peak arterial flow (PEAK), pulsatility index of arterial flow (PI), resistivity index of arterial flow (RI). Positive value: >0; negative value: <0.

In the left lower limb, low correlations (from 0.26 to 0.49) were observed between joint (both plantar flexion and dorsiflexion) movements and the values of mean velocity, peak, and resistivity index of the blood circulation of both tibial posterior and dorsal arteries were analyzed. There was a moderate correlation (from 0.50 to 0.69) between dorsiflexion and the left dorsal artery pulsatility index in the moments before and after dynamic evaluation, with significant values for the lower dorsiflexion and the lower pulsatility index (Table 3).

DISCUSSION

This study involved the evaluation and correlation of the range of motion of the ankle joint with the cutaneous surface temperature of the leg and the circulation of the posterior and dorsal tibial arteries before and after dynamic evaluation in which a series of motion repetitions were performed to verify joint mobility. A low correlation between the tibiotarsal range of motion and cutaneous surface temperature, and a moderate correlation between the range of motion and arterial blood circulation of the lower limb were identified.

Regarding the detrimental effects of diabetes on body structures, morphological changes in the tendons can be identified which cause decreased mobility of the ankle joint[4] and may impair the efficiency of muscle contraction, thereby interfering with movement stabilization and acceleration and consequently decreasing the functionality of these individuals[20]. The restriction of joint mobility interferes with the adequate contraction of the musculature and consequently with the blood circulation of the lower limb, with a decrease in the speed of blood flow[21], which corroborates the findings of the present study, in which reduced values of joint range of motion and arterial blood flow were observed.

The present study demonstrated a limitation in the range of articular movement of diabetic neuropathic individuals, as shown in the studies by Fernando et al[20]. that found a decrease in the range of ankle joint movement, resulting in a change in plantar distribution during gait, which can be identified as a risk factor for ulcers and lower quality of life in these patients.

Diabetic individuals often present with vascular calcification that increases arterial wall stiffness and systolic pressure[22], which is supported by the findings of the present study based on the pulsatility and resistivity data of the evaluated arteries. The lower the values of both indices, the slower the flow within the vessels, with the slower continuous flow being found in diabetic individuals[23].

The superficial cutaneous temperature of the skin of diabetic individuals is higher compared to that of healthy individuals due to an increase in heat emission caused by the thermoregulatory mechanisms present in the blood flow of cutaneous vessels, and being related to the presence of peripheral arterial disease[24].

The study by Weigert et al.[25] evaluated the body composition referring to the accumulation of fat in tissues in obese individuals and also observed a long time to change the skin surface temperature after resistance training. Body fat acts as a

Table 3. Results of correlation between range of motion and blood circulation of the left lower limb at the pre- (PRE) and post-immediate (POST) moments.

Left tibial	PRE				POST			
	L PF (r-value)	p	L DF (r-value)	p	L PF (r-value)	p	L DF (r-value)	p
MEAN	-0.333	0.152	-0.258	0.272	-0.271	0.248	-0.181	0.444
PEAK	-0.236	0.316	0.005	0.982	-0.047	0.845	0.065	0.786
PI	-0.015	0.950	0.332	0.153	0.420	0.065	0.325	0.163
RI	-0.133	0.575	0.049	0.838	0.047	0.842	0.144	0.545
Left dorsal	**L PF (r-value)**	**p**	**L DF (r-value)**	**p**	**L PF (r-value)**	**p**	**L DF (r-value)**	**p**
MEAN	-0.159	0.503	-0.132	0.579	-0.192	0.418	-0.096	0.688
PEAK	-0.264	0.260	0.283	0.227	-0.261	0.266	0.346	0.135
PI	-0.135	0.571	0.497*	0.026	-0.124	0.603	0.511*	0.021
RI	-0.219	0.353	0.327	0.159	-0.308	0.186	0.217	0.357

Values presented in correlation index (r-value) and p-value. Left plantar flexion (L PF), left dorsiflexion (L DF), left posterior tibial artery (LEFT TIBIAL), left dorsal artery (LEFT DORSAL), mean arterial flow velocity (MEAN), peak arterial flow (PEAK), pulsatility index of arterial flow (PI), resistivity index of arterial flow (RI). Positive value: >0; negative value: <0.

thermal insulator by reducing thermal conductivity and thus hindering heat exchange with the environment. Thus, the average surface temperature depends on the body fat[25]. The patients in the present study had a mean body mass index between 30 and 34.99, and were categorized as type I obesity. This may explain the results found in relation to the skin surface temperature, which did not change after the dynamic evaluation since it would take a longer time for the skin surface temperature to change, and the evaluation was performed immediately after exercise.

The results obtained in this study reveal a correlation between the tibiotarsal range of motion and peripheral blood circulation in diabetic individuals which interferes with the health of these individuals, thus reinforcing the importance of evaluating these parameters in clinical practice to preserve functionality and prevent related comorbidities throughout the disease.

CONCLUSION

The results of the present study point to a moderate correlation between the range of motion of the tibiotarsal joint and blood circulation and low temperature, demonstrating a reduction in the range of motion of the ankle and in the arterial blood flow of the lower limb of patients with diabetic neuropathy.

AUTHORS' CONTRIBUTIONS

CCZ: Conceptualization (equal), Data curation (equal), Writing – original draft (equal), Writing – review & editing (equal). **AG:** Data curation (equal), Formal Analysis (equal), Writing – review & editing (equal). **ATS:** Data curation (equal), Formal Analysis (equal). **GC:** Data curation (equal), Formal Analysis (equal). **RRJG:** Methodology (equal), Writing – review & editing (equal). **ECOG:** Conceptualization (equal), Writing – review & editing (equal).

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ORIGINAL ARTICLE

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Indoleamine-2,3-dioxygenase-related anti-inflammatory effects of 3-aminobenzamide and infliximab in experimental colitis

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SUMMARY

OBJECTIVE: This study aimed to investigate the presence of indoleamine-2,3-dioxygenase and bacterial translocation after the administration of 3-aminobenzamide and infliximab in the TNBS model of rat colitis.

METHODS: The study group was divided into five categories as follows: group 1: (control), group 2: colitis+saline, group 3: colitis+3-aminobenzamide, group 4: colitis+infliximab, and group 5: colitis+3-aminobenzamide+infliximab. Intestinal mesenteric cultures were incubated on specific agar media plates under aerobic and anaerobic conditions, bacterial translocation was evaluated and assessed as colony-forming units per gram of tissue. Colonic tissue samples were evaluated by Western blotting method to detect the presence of indoleamine-2,3-dioxygenase.

RESULTS: The results obtained were as follows: group 1: normal gut flora; group 2: eight of nine samples had bacterial translocation, of which six of them had positive indoleamine-2,3-dioxygenase protein; group 3: five of nine samples had bacterial translocation, of which seven of them had positive indoleamine-2,3-dioxygenase; group 4: three of nine samples had bacterial translocation, of which seven of them had positive indoleamine-2,3-dioxygenase; and group 5: only one sample had exact indoleamine-2,3-dioxygenase protein.

CONCLUSION: Altered expression of indoleamine-2,3-dioxygenase results in a lower bacterial translocation via infliximab compared with 3-aminobenzamide treatment. Combined treatments emphasized different approaches for the new molecules related to indoleamine-2,3-dioxygenase.

KEYWORDS: Inflammatory bowel disease. Ulcerative colitis. 3-Aminobenzamide. Infliximab. Indoleamine 2,3-dioxygenase.

INTRODUCTION

The drug 3-aminobenzamide (3-AB) is a pharmacological inhibitor of poly (ADP-ribose) polymerase-1 (PARP)[1]. Infliximab, another pharmacological agent, is a chimeric monoclonal antibody formed against tumor necrosis factor alfa (TNF-α)[2]. Although the etiology of inflammatory bowel disease (IBD) is still unknown, the pathophysiology is concentrated on the destructive activity of the reactive oxygen and nitrogen radicals and the excessive production of pro-inflammatory mediators[3].

Disruption of the intestinal homeostasis and tolerance toward the resident microbiota can be a major mechanism involved in the development of IBD. Depending on the response to luminal antigens, a controlled inflammation occurs and this is rapidly downregulated with the elimination of the pathogen. However, this balance is spoilt at IBD in favor of chronic inflammation[4]. Indoleamine-2,3-dioxygenase-1 (IDO, EC1.13.11.41), which catalyzes the first and limiting step of tryptophan catabolism, is thought to play a role in the control of intestinal inflammation; however, its role in the intestinal immunity has not been fully understood. However, in patients with IBD, the IDO enzyme was shown to be expressed above the normal from the biopsies of lesions[5,6]. IDO is also induced by IFN-γ, which is both natural and the strongest inducer at the adaptive immune response and, though still under discussion, by TNF-α[2,7].

In this study, we aimed to compare the protein expression of rate-limiting enzyme IDO of the kynurenine pathway and the differences in bacterial translocation (BT) in the TNBS

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model of rat colitis treated with both PARP enzyme inhibitor and the TNF-α receptor blocker infliximab.

METHODS

This study was verified by Ankara Training and Research Hospital Education, Planning, Coordination and Ethic Committee (AEAH.2007.0227) and performed in accordance with the National Institutes of Health guidelines for the care and handling of animals.

Study protocol

The male Wistar albino rats weighing between 250 and 300 g were collected from Ankara Training and Research Hospital (Ankara, Turkey). Rats were divided into five separate groups and kept in five separate cages with conventional housing system at 12-h light/dark cycles till the end of the study period. Male rats were randomly divided into five groups as follows: group 1: sham+saline (n=8), group 2: colitis+saline (n=9), group 3: colitis+3-AB (n=9), group 4: colitis+infliximab (n=9), and group 5: colitis+3-AB+infliximab (n=7). All rats were fed with standard food, water was ad libitum, and food was stopped 24 h before the rectal administration of TNBS or saline to the subjects. The colitis model was formed with a mixture of 0.8 ml of 5% (40 mg) TNBS (Sigma Chemical Co., St. Louis, MO, USA) and 0.4 ml of absolute ethanol according to previous literature[8]. To form colitis, 1.2 ml of TNBS–ethanol (GATA Biochemistry Laboratory, Ankara, Turkey) mixture was then given rectally; slowly administered by using polyethylene catheter 0.7 mm in diameter 8 cm forward into the anus; subjects were hold upside down for 30 s so that the substance spreads on the colon surface[9].

A dose of 10 mg/ml of 3-AB (Sigma, USA) and 10 mg/kg of infliximab (Sigma) was prepared to administrate to the treatment group and protected at +4°C until the experiment.

Group 1 and group 2 injected saline (1 ml., i.p. every 12 h), group 3 treated with 3-AB (10 mg/kg, i.p. every 12 h), group 4 received infliximab (10 mg/kg, i.p. every 24 h), and group 5 received both 3-AB and infliximab (3-AB 10 mg/kg, i.p. every 12 h, infliximab 10 mg/kg, i.p. every 24 h) 24 h before colitis was formed. A dose of 1.2 ml of saline at room temperature was rectally administered to group 1. After 7 days, rats were sacrificed under general anesthesia by cervical dislocation sacrification procedure. Colon was resected by laparotomy with a median incision in the supine position, in the manner to include healthy borders 2 cm to the proximal and distal to the segment with colitis in group 2 and group 3, which included approximately 6 cm segment of rectum. The part of the distal rectum included 6 cm segment of the rectum was removed in group 1. Macroscopically colon segments with mucosal focal hyperemia, ulceration, and thickening on colon wall were accepted as segment with colitis[10,11]. It was also sent to the pathological lab for microscopic examination and the next 3-cm colonic segment was placed into the cold chain for biochemical studies.

Those slides for histopathological examination were stained by hematoxylin and eosin (H&E). The total histological score was found by adding the epithelium and infiltration scores[12].

Tissue samples were tested daily by Western blotting method for the detection of IDO. Total protein concentration was measured by a commercial Pierce BCA Protein assay kit using bicinchoninic acid (BCA).

Mesenteric tissue complex was excised under sterile conditions, transferred into the test tubes containing thioglycolate broth medium (Merck, Germany), and cultured to investigate the BT.

Statistical analysis

Analysis of data was done with SPSS version 17 package program. Descriptive statistics were given as mean value±standard deviation. Differences in continuous variables and significance of the difference in terms of averages between groups were analyzed using the Mann-Whitney U test. Categorical variables were analyzed using chi-square tests. Results for $p<0.05$ were accepted statistically significant.

RESULTS

Histopathological finding among groups

The total scores of histopathology are shown in Figure 1. The pathological scores of groups 3 and 4 were statistically significantly decreased compared to group 2 ($p<0.05$).

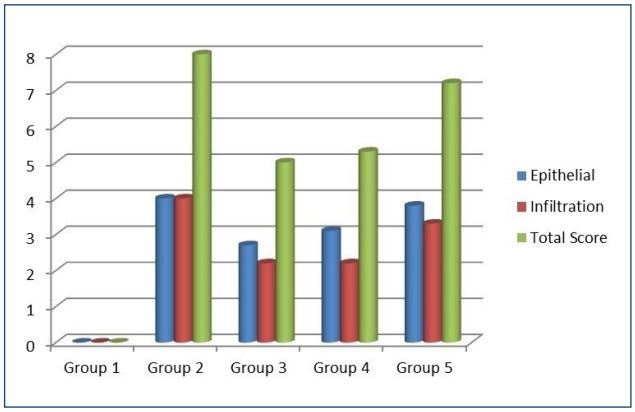

Figure 1. Evaluation of the histopathological findings.

Association between bacterial translocation and indoleamine-2,3-dioxygenase expression

The total bacterial amounts (cfu/ml) cultured under aerobic and anaerobic conditions are presented in Table 1.

The chemiluminescence images of polyvinylidene difluoride (PVDF) membranes belonging to β-actin and IDO protein bands in all groups were detected. The difference between the groups for the culture results and IDO expression was evaluated as percentage in order to apply the results easily for clinical interpretations. Overall, the culture results and IDO expression were detected in group 1 (normal gut flora), but BT and IDO were not exactly detectable in all samples. In group 2, 8 (88.9%) of the 9 samples had BT, of which 6 (66.7%) samples had positive IDO protein. In group 3, 5 (55.6%) of the 9 samples had BT, of which 7 (77.8%) samples had positive IDO protein. In group 4, 3 (33.3%) of the 9 samples had BT and IDO protein was positive in 7 (77.8%) of these samples. In group 5, no BT was found and none of the samples had exact IDO protein; only one might had IDO protein, which we considered positive. Finally, the relations between BT percentage (%) and IDO expression (%) are presented in Table 2.

DISCUSSION

This study investigated the effects of TNF-α and PARP inhibitors on IDO-induced BT depletion in an experimental IBD model. IDO expression, the major enzyme in tryptophan metabolism, was measured and the BT formation was evaluated in TNBS-induced model. The evaluation of damage in the colonic specimens was mainly done histologically, and the characterization of the intestine microbiota was performed by microbiological methods. While discussing the relationship of BT and IDO expression as percentage results, we observed either similar or contrary results to literature. Both treatments decreased the BT%, but the combination therapy was confusingly the best treatment for BT. The major results of the study were that BT was inhibited by using TNF-α and PARP inhibitors and the expression of IDO was lower in those groups. However, a remarkable decrease in IDO expression was observed in both 3-AB and infliximab combined treated group.

Although gut-associated microbial community reveals anaerobic and aerobic bacteria in healthy subjects, intestinal inflammation is associated with the disturbance of the microbiota and often includes an increased prevalence of

Table 1. Total bacterial amount (cfu/mL) cultured under aerobic and anaerobic conditions.

	Groups					p-value
	1	2	3	4	5	
	Mean±SD Median (min-max)	Mean±SD Median (min-max)	Mean±SD Median (min-max)	Mean±SD Median (min-max)	Mean±SD Median (min-max)	
Aerobic conditions (cfu/ml)	24.50±6.25 23.00 (15–35)	34.67±15.13 32.00 (13–60)	28.11±27.09 15.00 (6–80)	38.11±29.01 33.00 (7–86)	27.29±19.57 19.00 (4–60)	(0.615) NS
Anaerobic conditions (cfu/ml)	26.13±19.11 16.00 (11–58)	34.00±51.17 13.00 (2–154)	14.56±9.26 11.00 (4–34)	47.78±55.31 24.00 (3–171)	43.00±38.54 31.00 (6–120)	(0.242) NS
Total (cfu/ml)	50.63±24.80 38.50 (31–93)	68.56±56.12 47.00 (18–202)	43.78±32.15 40.00 (10–100)	85.56±69.28 61.00 (11–204)	70.29±49.61 39.00 (25–152)	(0.560) NS

Group 1: sham+saline (n=8), Group 2: colitis+saline (n=9), Group 3: colitis+3-AB (n=9), Group 4: colitis+infliximab (n=9), Group 5: colitis+ 3-AB+infliximab (n=7).

Table 2. Association between bacterial translocation (%) and indoleamine-2,3-dioxygenase expression (%) among groups.

		Groups (n, %)					p-value
		1	2	3	4	5	
BT	None	8 (100%)	1 (11.1%)	4 (44.4%)	6 (66.7%)	7 (100%)	<0.001
	Positive	0 (0.0%)	8 (88.9%)	5 (55.6%)	3 (33.3%)	0 (0.0%)	
IDO	None	8 (100%)	3 (33.3%)	2 (22.2%)	2 (22.2%)	6 (85.7%)	0.001
	Positive	0 (0.0%)	6 (66.7%)	7 (77.8%)	7 (77.8%)	1 (14.3%)	

Group 1: sham+saline (n=8), Group 2: colitis+saline (n=9), Group 3: colitis+3-AB (n=9), Group 4: colitis+infliximab (n=9), Group 5: colitis+ 3-AB+infliximab (n=7). BT: Bacterial translocation; IDO expression.

facultative anaerobic bacteria, which can further exacerbate inflammation[13]. Usage of anti-inflammatory and immunomodulatory agents can control mucosal inflammation and change clinical course of the disease by preventing complications[14]. During inflammation, one excepted idea is the electron acceptors generated as by-products of the host inflammatory response that can feed selectively facultative anaerobic bacteria. Also, excessive secretion of T-helper cells and chemokines can cause to degenerate tolerance and immunoregulation toward antigens by controlling T_{H1}-cell proliferation[15]. IDO facilitates the formation of suitable immune response by affecting the balance between immune tolerance and attack. It is also induced by TNF-α, IFN-α/β, and IL-10 which are produced during infections[16,17].

Generally, TNF-α, which is abundantly expressed from the intestines of IBD patients, is a pro-inflammatory cytokine that plays an important role in pathogenesis of IBD and contributes to enteritis as an induction of apoptosis in villous epithelial cells, disruption of the epithelial barrier, and secretion of chemokines in intestinal epithelial cells. Infliximab decreases CD25 expression and inhibits the release of IFN-γ, IL-13, IL-17A, and TNF in the CD4+ and CD8+ T-cell population[18]. In our study, 3-AB- and infliximab-treated groups had increased expression of IDO and reduced bacterial growth, which was due to an increased degradation of tryptophan. However, in infliximab-treated group, the expectation was the inhibition of TNF-α effect, but we observed 77.8% of IDO expression and had better results for BT%. The alterations in the enzyme activity by TNF-α inhibitor and PARP inhibitor might be used as an indicator in deciding whether to continue the pharmacotherapy and in determining the effect of drug. In the treated groups, the cause of an increased expression of IDO might be due to inadequate doses or inadequate routine treatment follow-up. Ciorba et al. suggested that increasing IDO expression within the intestine may have the therapeutic capacity to abrogate colitis[19]. In our study, IDO expressions were high in treatment groups 3 and 4 compared to colitis group and had lower histopathological scores with decreased BT%.

One of the main courses of IDO expression is to act as an effective agent preventing bacterial and/or viral infections of the closed-body fields such as ductus epididymis by decreasing the production of tryptophan[11]. BT is the transmission of vital endogen bacteria from intestinal lumen to mesenteric tissues and other gastrointestinal organs. There is a positive correlation between the severity of colitis and the BT. In a recent study, in group 2 (i.e., colitis group), a higher BT (88.9%) was observed. Both treatments could improve the BT%, but the combination of treatments was fully potent. The presence of IDO activity and depletion of local tryptophan can cause growth arrest of several tryptophan-dependent microorganisms as a local anti-infectious agent[20]. However, a conflicting result was found in group 5, with only one detectable IDO expression and nearly no BT, indicating that the synergistic effect of combined treatments might be efficient to yield the use of the enzyme to avoid BT. In another study, the inhibition of this enzyme worsen the disease severity, suggesting that it acts as a natural blocker in limiting colitis. In our study, we also had a higher score for histopathology, supporting the severity of disease in group 5.

One possible role of IDO in the endothelium may be as a free radical scavenger due to its use of superoxide anion. IDO may serve as an important antioxidant role[21]. Park et al. suggested that the IDO-mediated depletion of tryptophan and subsequent accumulation of active metabolites may have neuroprotective effects on ischemic injury to prevent hippocampal neuronal cell death[22].

CONCLUSIONS

Our study reports about the effects of TNF-α and PARP inhibitors on IDO-induced BT depletion in an experimental IBD model. The major results of the study reveal that BT was inhibited by using TNF-α and PARP inhibitors and the expression of IDO was decreased. This study can lead to the development of new molecules for pharmacological inhibition of IDO activity in several clinical settings. The detailed metabolic pathway studies for IDO will enable to design and develop new molecules for the pharmacological inhibition or the activation of IDO activity in several clinical settings.

AUTHORS' CONTRIBUTIONS

EM: Conceptualization, Data curation, Formal Analysis, Investigation, Methodology, Project administration, Resources, Visualization, Writing – original draft. **SA:** Data curation, Resources, Supervision, Visualization. **OUA:** Data curation, Resources, Software, Supervision. **DS:** Formal Analysis, Resources, Software, Supervision. **GA:** Resources, Software, Supervision. **AE:** Formal Analysis, Funding acquisition, Writing – review & editing. **ASD:** Conceptualization, Investigation, Methodology, Project administration, Validation, Writing – review & editing. **YO:** Conceptualization, Investigation, Methodology, Validation, Writing – review & editing. **MD:** Methodology, Validation, Writing – review & editing.

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ORIGINAL ARTICLE

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Intra- and inter-examiner reliability of digital images of skin donor areas in burns

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SUMMARY

OBJECTIVE: The main objective of this study was to evaluate intra- and inter-rater reliability in the analysis of digital images of donor areas for skin in burn patients using the CaPAS plugin in the ImageJ®.

METHODS: Donor sites were reviewed by two independent reviewers in duplicate. The capture of images was standardized on the same device and distance (with a millimeter ruler), without a flash. The evaluators were trained to capture the images and use the plugin.

RESULTS: We selected 70 images from donor areas, from men and women between 18 and 60 years old. In the analysis of intra-examiner reliability, eight of the nine variables exhibited excellent reliability (0.985–0.998) and one (entropy) exhibited good reliability (0.525). The same was true for the inter-examiner analysis: excellent reliability for eight variables (0.824–0.993) and good reliability for entropy (0.501).

CONCLUSIONS: The CaPAS plugin has proven to be a reliable tool for use in research in skin donor areas in burns, as demonstrated by its excellent intra- and inter-examiner reliability values. This is a pioneering study in the quantitative assessment of skin donor areas in burn patients using the CaPAS plugin.

KEYWORDS: Reproducibility of results. Data accuracy. Burns.

INTRODUCTION

Digital photographs have been used for skin analysis in research with an interest in more reliable investigations of tissue characteristics[1]. Creating new tools to quantify and validate these images is a challenge[2], since a subjective analysis only provides qualitative data that are difficult to reproduce[3]. The clinical evaluation of the burned patient (both donor and recipient areas) is routinely performed qualitatively through direct inspection[4].

The easy-to-use ImageJ® public domain software developed in Java programming at the U.S. National Institutes of Health is a well-known health tool for image processing that has also been used for the assessment of skin lesions and can, therefore, be very useful in the evaluation of burn patients[5].

The CaPAS plugin was designed for carotid plaque analysis and was developed for the quantitative evaluation of videodensiometric images of atherosclerosis through computational measurements. This tool is sensitive to grayscale changes in cholesterol, collagen, and calcium, enabling the differentiation of groups[6], patients with and without symptoms have a different plaque composition[7].

The use of the CaPAS plugin in burn patients was reported in a study that analyzed images of skin donor areas (e.g., thigh and scalp) in two groups (i.e., sham and intervention) to investigate re-epithelialization[8]. The plugin made it possible to quantify the images and differentiate the texture of the re-epithelialized tissue. However, no plugin properties or variables were evaluated.

The aim of this study was to evaluate intra- and inter-rater reliability in the analysis of images of skin donor areas in burn patients using the CaPAS plugin implemented in ImageJ®.

METHODS

A total of 70 images (32 scalp and 38 thigh) of 70 patients were taken from skin donor areas of burn patients admitted to the emergency unit of the hospital of the Ribeirão Preto School of Medicine. One was randomly selected using a simple lottery

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system for analysis by two evaluators, according to previous health reliability studies[9].

Images were taken using a 12-megapixel digital camera (DSC-W510, Sony, Manaus, Brazil) with 72 dpi. The images were taken without flash with the camera perpendicular to the area to be photographed at a standardized distance of 30 cm apart (determined using a millimeter ruler) on the same plane as the sample. This was performed by two independent evaluators who had undergone training for the use of the software. All images were analyzed twice, with a 1-week interval between evaluations. The evaluation and digitization of the analyses followed the same order (1–70) for both evaluators. The image evaluation procedure is described in Figure 1.

Outline the area of interest with the computer mouse. Select the ANALYSIS icon. Then, the graph with the nine measurements will be generated: Count: number of pixels within the area; Average: average pixel value calculated on the pixels in the selected region; Standard Deviation: of the gray values of the pixel in the selected region; Skewness: asymmetry (when positive, asymmetry is toward the dark side; when negative, asymmetry is toward the light side); Kurtosis: graph symmetry, Energy: angular value, when the elements of the co-occurrence matrix are very unequal; Inertia: intensity contrast between a pixel and its neighbor; Entropy: degree of uncertainty in the uniformity of the selected region (its value increases when homogeneity reduces); and Homogeneity: dissimilarity and contrast.

These measurements are represented by texture parameters involving mean and standard deviation, and grayscale parameters (i.e., entropy, energy, and homogeneity). The mean gray level (MGL) represents the average grayscale value of the pixels in the region of interest on a scale of 256 shades of gray, with zero being the darkest possible tone (hypoechoic) and 255 the lightest possible tone (hyperechoic)[6].

Intra- and inter-rater reliability were determined using the intraclass correlation coefficient (ICC2,1), with the calculation of the 95% confidence interval, standard error of measurement (SEM), and minimal detectable change (MDC). ICC values were interpreted based on Weir[10]: 1.00–0.81 indicates excellent reliability; 0.80–0.61 indicates very good reliability; 0.60–0.41 indicates good reliability; 0.40–0.21 indicates reasonable reliability; and 0.20–0.00 indicates poor reliability. The statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS), version 17.0 (Chicago, IL, USA).

RESULTS

A total of 70 digital photographic images of 70 patients of both sexes between the ages of 18 and 60 years were evaluated. The mean values are described in Table 1.

Table 2 shows intra- and inter-rater ICC values. Intra-rater ICC values ranged from 0.985 to 0.998 (excellent reliability) for eight of the nine variables. The intra-rater ICC for "entropy" was 0.525 and inter-rater ICC for "entropy" was 0.501, thus good reliability was found for entropy, while excellent reliability was found for other variables (ICC=0.824–0.993).

DISCUSSION

The evaluation of burns is commonly performed by local visual inspection, being a quick and easy method, but subjective, not being the most reliable or accurate[11,12], as it strongly depends on the experience of the evaluator.

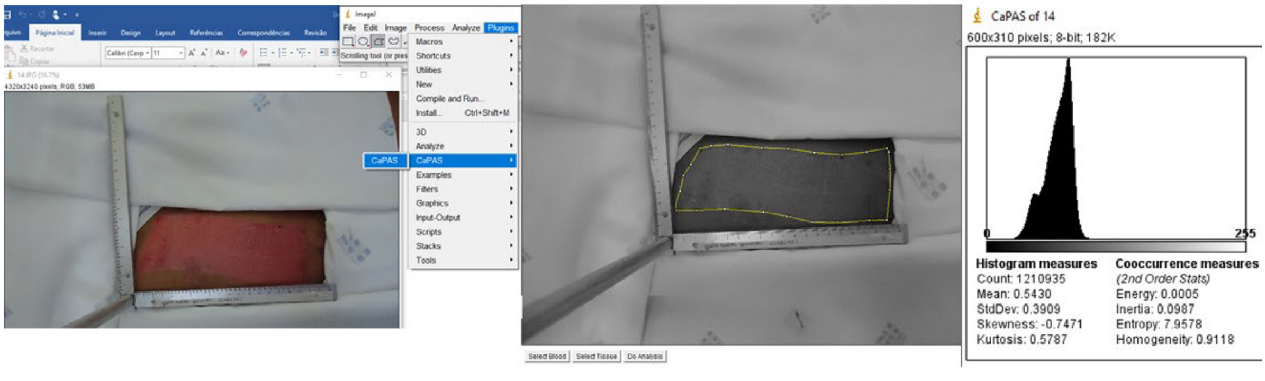

Figure 1. Importing the image in the File icon. After inserting the image, click twice on the CaPAS icon. At this moment, the image will appear in grayscale.

Table 1. Description of the mean of the variables by both evaluators.

Variables	Evaluator 1		Evaluator 2	
	Test	Retest	Test	Retest
Count	490,280 (361,584)	480,188 (369,297)	447,302 (342,365)	453,803 (349,364)
Mean	0.73 (0.23)	0.73 (0.22)	0.72 (0.23)	0.72 (0.23)
Standard deviation	0.53 (0.16)	0.52 (0.16)	0.52 (0.17)	0.52 (0.16)
Skewness	-0.76 (0.03)	-0.76 (0.02)	-0.75 (0.05)	-0.76 (0.03)
Kurtosis	0.60 (0.05)	0.60 (0.05)	0.62 (0.16)	0.60 (0.05)
Energy	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
Inertia	0.04 (0.03)	0.04 (0.03)	0.05 (0.07)	0.05 (0.04)
Entropy	8.99 (0.60)	9.00 (0.61)	8.97 (0.64)	8.86 (1.18)
Homogeneity	0.95 (0.03)	0.95 (0.03)	0.95 (0.03)	0.95 (0.03)

Data expressed as mean (standard deviation).

Table 2. Summary of intra- and inter-examiner reliability obtained.

Variables	ICC	95%CI	SEM (%)	MDC (pixels)
Intra-rater				
Count	0.997	0.995–0.998	4.13	55,481.56
Mean	0.998	0.996–0.998	1.40	0.03
Standard deviation	0.997	0.996–0.998	1.66	0.02
Skewness	0.991	0.986–0.995	0.38	0.01
Kurtosis	0.992	0.987–0.995	0.86	0.01
Energy	0.989	0.982–0.993	8.93	0.00
Inertia	0.980	0.967–0.987	11.28	0.02
Entropy	0.525	0.236–0.705	4.67	1.17
Homogeneity	0.985	0.976–0.991	0.41	0.01
Inter-rater				
Count	0.983	0.972–0.989	10.03	129,864.35
Mean	0.993	0.989–0.996	2.68	0.05
Standard deviation	0.993	0.988–0.995	2.59	0.04
Skewness	0.985	0.975–0.990	0.48	0.01
Kurtosis	0.987	0.980–0.992	1.09	0.02
Energy	0.824	0.717–0.891	35.53	0.00
Inertia	0.895	0.831–0.935	26.35	0.04
Entropy	0.501	0.197–0.690	7.11	1.76
Homogeneity	0.903	0.843–0.939	1.06	0.03

ICC: intraclass correlation coefficient; CI: confidence interval; SEM: standard error of measurement (% score); MDC: minimal detectable change (% score).

The use of digital photographs is a viable alternative to overcome this limitation. It has been used in research on skin erythema[13], skin cancer[14], and vocal folds[15], seeking to quantify differences between treatments and/or textures, and is also used in clinical practice.

Skin assessment can be performed using instruments such as the Vancouver Scar Scale and the Patient and Observer Scar Assessment Scale[16], which require training and specific knowledge of their psychometric properties. Another possibility is the laser Doppler, which is expensive and not always available in burn centers[17].

Hop et al.[18] highlighted the need for more accurate assessments of burns, as the cost of hospitalizations and surgeries is very high and optimal treatment requires an accurate diagnosis. As demonstrated in this study, the use of quantitative assessment based on digital photographs is highly reproducible and meets the requirements for assessing skin donor areas for grafts in burn patients.

As this is a reliability study, whose objective was to determine the reproducibility of using the CaPAS plugin as a tool to assess the skin surface, the images used were standardized in a skin donor area. The removal of the skin was always performed with the same thickness, dermatome, and defined edges, allowing to differentiate the intact skin. Excellent intra- and inter-examiner reliability was found for eight of the nine variables of the CaPAS plugin and very good reliability for the variable "entropy." The lower reliability of this variable may be due to its greater sensitivity when performed by different evaluators, as it depends on the demarcation of the image and the grayscale pixel values. With lighter or darker pixels, an important difference in variables can occur even with a difference in demarcation of millimeters between evaluators.

In a study that investigated the re-epithelialization of the donor area in burns, the inertia and homogeneity variables were able to differentiate different groups[8]. In this research, it is noteworthy, therefore, that both variables showed excellent intra- and inter-observer correlation for the same population, making their use reliable and reproducible as a quantitative way of evaluating the donor area.

Reliability studies consider ICC values >0.40 acceptable[19]. In this research, the intra- and inter-examiner ICC values found are all acceptable for reliability. Originally colored images are transformed into black and white during the plugin execution, which may explain the small divergence between evaluators regarding the demarcation of the selected area's borders. The good intra-examiner correlation for this item validates those of the plugin, as the intensity of a pixel is more important than its homogeneity.

The statistical value of energy increases when the co-occurring matrix elements are very unequal, which means large differences in texture patterns within the skin removal area. Homogeneity measurements reflect the level of roughness of the donor area. Thus, when homogeneity is high, the homogeneous distribution of texture patterns is found within the selected skin donor area, which means irregular but homogeneous pixel tones.

The high "count" values, which correspond to the pixel area, obtained in the intra- and inter-rater analyses result from the calculations performed, in which the area is multiplied by millimeters squared (mm^2) of pixels, which requires the use of a millimeter ruler on the same plane as the area of interest when the digital image is captured.

In reliability studies, it is important to establish the amount of error inherent to a given measurement method[20]. The SEM indicates errors when measuring a particular variable with an assessment tool, i.e., it is an indication of the accuracy of a score. Regarding the MDC, Weir[10] emphasized that it is important to be aware of the minimum difference in scores of an assessment instrument between reevaluations.

The present study offers a promising analysis of the quantitative evaluation of skin donor areas in burn patients, capable of detecting small variations in the skin surface. This method is reliable and allows for more rigorous evaluation of these patients. In fact, most studies involving the evaluation of burned skin or donor area involve qualitative analysis, whose results may be imprecise, as they depend on the evaluator's perception, experience, and criteria. The analysis of digital photographs using the CaPAS plugin also allows the monitoring of the healing process and the effect of different treatments in both research and clinical practice.

The reliability of this tool allows its use in this population, as well as in other populations in which skin repair is investigated. ImageJ® software is easy-to-use and public domain, so it is free and only a digital photograph is required to run the plugin, in addition to providing fast results, making it an extremely valuable assessment tool for burn patients.

CONCLUSION

The CaPAS plugin implemented in ImageJ® software proved to be a reliable tool for use in research and clinical practice involving the analysis of skin donor areas in burn victims, as demonstrated by the excellent intra- and inter-rater reliability values.

AUTHORS' CONTRIBUTIONS

FFOA: Conceptualization, Investigation, Formal Analysis, Supervision, Writing – original draft, Validation, Visualization, Project administration, Writing – review & editing. **RCG:** Investigation, Formal Analysis, Writing – original draft, Validation, Visualization, Project administration, Writing – review & editing. **NCSB:** Data curation. **LOMJ:** Methodology, Resources, Software, Validation, Visualization, Project administration, Writing – review & editing. **JAFJ:** Methodology, Validation, Visualization, Project administration, Writing – review & editing. **ECOG:** Supervision, Validation, Visualization, Project administration, Writing – review & editing. **RRJG:** Conceptualization, Formal Analysis, Funding acquisition, Methodology, Supervision, Validation, Visualization, Project administration, Writing – review & editing.

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ORIGINAL ARTICLE

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Clinical and radiological characteristics of pulmonary actinomycosis mimicking lung malignancy

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SUMMARY

INTRODUCTION: Pulmonary actinomycosis, clinically and radiologically, mimics abscess, tuberculosis, and lung malignancy, resulting in misdiagnosis or delay in diagnosis. In this study, we analyzed the clinicoradiological features of pulmonary actinomycosis, the presence of any differences between clinical prediagnosis and radiological diagnosis, and whether imaging modalities help distinguish pulmonary actinomycosis from lung cancer.

METHODS: A total of 22 patients who had a histopathological diagnosis of actinomycosis in a tertiary health center participated in this study. Of these, 14 had positron-emission tomography/computed tomography.

RESULTS: In all, 81.8% of the patients were males. The diagnostic procedures employed for the diagnosis of actinomycosis were surgery in 54.5% of patients, fiberoptic bronchoscopy in 36.4% of patients, and rigid bronchoscopy in 9.1% of patients. Radiological and clinical prediagnosis showed malignancy in 31.8 and 40.9% of patients, respectively. The mean of the maximum standardized uptake value was 6.33±3.6 on positron-emission tomography/computed tomography. Kappa compliance analysis revealed that clinical and radiological diagnoses were significantly compatible with each other and that radiological pre-diagnoses were not superior to clinical diagnoses (κ=0.701 and p<0.001).

CONCLUSION: Pulmonary actinomycosis shows high metabolic uptake in positron-emission tomography/computed tomography, and this may mislead clinicians for a diagnosis of malignancy. Our results suggest that positron-emission tomography/computed tomography does not help distinguish pulmonary actinomycosis from lung malignancy and does not provide a clear diagnostic benefit to the clinician, so pathological diagnosis is necessary.

KEYWORDS: Actinomycosis. Neoplasms. Tomography. Positron-emission tomography.

INTRODUCTION

Actinomycosis is a disease caused by Gram-positive anaerobic, non-acid-resistant bacteria belonging to the Actinomycetaceae family. These bacteria are present in normal human flora and are often isolated from mucosal surfaces of the oral cavity, gastrointestinal tract, and female genital tract[1].

Actinomycosis often involves the cervicofacial region but can involve all systems in the body. Unusual areas of involvement include the pulmonary tract, abdominopelvic region, central nervous system, skin, heart, and genitourinary tract. Infection occurs when *Actinomyces* in normal flora invade the mucosa that gets damaged due to various predisposing factors. Predisposing factors include poor oral hygiene, gingival disorders and surgery, diabetes mellitus (DM), and chronic respiratory disorders such as bronchiectasis[2].

Approximately 15% of cases with actinomycosis have pulmonary involvement. When lungs are involved, diagnosis of actinomycosis is difficult since it clinically and radiologically mimics abscess, tuberculosis, and lung cancer, resulting in misdiagnosis or delay in the diagnosis[3]. Pulmonary actinomycosis (PA) usually presents with cough, fever, chest pain, and, rarely, hemoptysis[2]. PA can present with lung consolidation, nodules, cavity formation, or a mass in the lung, mimicking lung cancer. It is often difficult to distinguish actinomycosis from malignancy due to its nonspecific clinical, radiological, and laboratory findings. Recent studies have reported that PA cases are often clinically prediagnosed as pulmonary malignancy[4,5,6,7]. Actinomycosis results in intense hypermetabolic regions in positron-emission tomography/computed tomography (PET-CT), as in malignancy[8].

In this study, we analyzed the clinicoradiological features of PA, the presence of any differences between clinical prediagnosis and radiological (PET-CT, thorax CT) diagnosis, and whether imaging modalities help distinguish PA from lung cancer.

METHODS

This study included a total of 22 patients who had a histopathological diagnosis of actinomycosis in a tertiary health center between

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2014 and 2019. Of them, 14 patients had PET-CT. The symptoms, comorbidities, predisposing factors, posteroanterior chest X-ray (CXR) findings, and the most common clinical prediagnoses, CT and/or PET-CT findings and radiological pre-diagnoses, the maximum standardized uptake values (SUV_{max}) of the lesion on PET-CT, diagnostic methods, and fiberoptic bronchoscopy (FOB) findings of the patients were obtained from the medical records of the hospital. Institutional Medical Education Board reviewed and approved the protocol of this retrospective study (approval date February 12, 2019; decree no.: 617).

The data were analyzed with the SPSS version 26.0 (IBM Corp., NY, USA) package program. Categorical variables were presented as percent and frequencies. The compliance of continuous variables to normal distribution was analyzed with the Shapiro-Wilk test. Continuous variables with a normal distribution were presented as mean±standard deviation, and continuous variables without a normal distribution were presented as median and interquartile range. Compatibility of radiological and clinical diagnoses was studied with Cohen's kappa analysis. All p-values were two-tailed and $p<0.05$ was considered statistically significant.

RESULTS

A total of 18 (81.8%) male and 4 (18.2%) female cases were included in the study. The median age of all patients was 53 years (interquartile range=48.5–67.75 years). Eight (36.4%) patients were current or former smokers. The median smoking pack-year was 20.5 (interquartile range=20–37.5 pack-years) (Table 1).

Analysis of the comorbid conditions and predisposing factors of the patients revealed that 10 (45.4%) patients had cystic bronchiectasis and a history of surgery due to bronchiectasis, 5 (22.7%) patients had DM, 4 (18.2%) patients had hypertension, 2 (9%) patients had surgery due to lung cancer, and 1 (4.5%) patient had tooth extraction previously.

The most frequent symptom in our patients [15 (68.2%)] was cough. The frequencies of the symptoms are shown in Table 1.

The diagnostic procedures employed for the diagnosis of actinomycosis were surgery in 12 (54.5%) patients, FOB in 8 (36.4%) patients, and rigid bronchoscopy in 2 (9.1%) patients. In FOB plus rigid bronchoscopy examinations (n=10), more than one finding was observed in each patient. These findings were narrowing of the bronchus by external pressure (30%), irregular mucosa (50%), granulation tissue (20%), endobronchial lesion (EBL) at the site of the surgical stump (20%), suture material at the site of the surgical stump (20%), and aspirated foreign body (a piece of bone) (20%).

Table 1. Demographic and clinical characteristics of the patients.

Male gender, n (%)	18 (81.8)
Age, years, median (IQR)	53 (48.5–67.75)
Smoking history, n (%)	8 (36.4)
Diabetes mellitus, n (%)	5 (22.7)
Hypertension, n (%)	4 (18.2)
Surgery due cystic bronchiectasis, n (%)	10 (45.4)
Surgery due to lung malignancy, n (%)	2 (9)
Tooth extraction, n (%)	1 (4.5)
Cough, n (%)	15 (68.2)
Sputum production, n (%)	8 (36.4)
Dyspnea, n (%)	8 (36.4)
Hemoptysis, n (%)	8 (36.4)
Back chest pain, n (%)	5 (22.7)
Weight loss, n (%)	4 (18.2)
Fever, n (%)	4 (18.2)
Foul breath, n (%)	1 (4.5)

IQR: interquartile range.

A total of 19 (86.4%) patients had CXR. Of these, in 7 (36.8%) patients, CXR was interpreted as normal. CXR findings are shown in Table 2. The lesions were located in the left lower lobe in 8 (36.4%) patients, right upper lobe in 5 (22.7%) patients, right lower lobe in 5 (22.7%) patients, right middle lobe in 2 (9.2%) patients, left upper lobe in 1 (4.5%) patient, and left lingular segment 1 (4.5%) patient on thorax CT (Table 2).

The most frequent finding on thorax CT was the presence of a mass, which is evident in 7 (31.8%) patients. The other findings were cavity (n=4, 18.2%), lymphadenopathy (n=7, 31.8%), consolidation (n=3, 13.6%), effusion (n=3, 13.6%), infected bronchiectasis (n=3, 13.6%), nodule (n=2, 9.1%), EBL (n=2, 9.1%), atelectasis (n=2, 9.1%), and ground-glass appearance (n=1, 4.5%).

A total of 14 (63.6%) patients had PET-CT. The findings on PET-CT were as follows: collapse consolidation in 4 (28.6%) patients, EBL in 3 (21.4%) patients, mass in 3 (21.4%) patients, nodule in 2 (14.3%) patients, and cavity in 2 (14.3%) patients (Table 2). The highest SUV_{max} on PET-CT was 11.77, and the mean SUV_{max} was 6.33±3.6.

The radiological prediagnosis was malignancy in 7 (31.8%) patients, pneumonia in 7 (31.8%) patients, infected bronchiectasis in 4 (18.2%) patients, benign lesions in 3 (13.6%) patients, and a recurrent malignant lesion in 1 (4.5%) patient. The clinical diagnoses of the patients were as follows: malignancy in 9 (40.9%) patients, pneumonia in 5 (22.7%) patients, infected bronchiectasis in 4 (18.2%) patients, recurrent malignant lesion in 2 (9.1%) patients, tuberculosis in 1 (4.5%) patient, and

empyema in 1 (4.5%) patient. The kappa compliance analysis determined that clinical and radiological diagnoses were statistically significantly compatible with each other, and radiological prediagnoses did not show superiority over clinical diagnoses (κ=0.701 and p<0.001). Table 3 shows the compliance analysis of clinical and radiological diagnoses.

In this study, malignancy was reported in 7 (31.8%) patients as the radiological pre-diagnosis, and a pre-diagnosis of recurrent malignancy was reported in 1 (4.5%) patient, while the clinical diagnosis was malignancy in 9 (40.9%) patients and recurrent malignancy in 2 (9%) patients. Kappa compliance analysis revealed that clinical and radiological diagnoses were statistically significantly compatible with each other, and radiological pre-diagnoses were not superior to clinical diagnoses (κ=0.701 and p<0.001).

Table 2. Radiological findings.

Chest radiography findings (n=19), n (%)	
Normal	7 (36.8)
Effusion	4 (21.1)
Cystic bronchiectasis	4 (21.1)
Infiltration	2 (10.5)
Cavity	1 (5.3)
Consolidation	1 (5.3)
Site of the lesion on CT (n=22), n (%)	
Left lower lobe	8 (36.4)
Right upper lobe	5 (22.7)
Right lower lobe	5 (22.7)
Right middle lobe	2 (9.2)
Left lingular segment	1 (4.5)
Left upper lobe	1 (4.5)
Lesion defined on PET-CT (n=14), n (%)	
Collapse-consolidation	4 (28.6)
Endobronchial lesion	3 (21.4)
Mass	3 (21.4)
Nodule	2 (14.3)
Cavity	2 (14.3)

PET-CT: positron-emission tomography/computed tomography.

DISCUSSION

Our study analyzed the past 5-year cases with a histopathological diagnosis of PA in a tertiary Pulmonary Training and Education Hospital, and as far as we know, it is the largest study performed on PA cases in a single institution in our country.

Actinomycosis is a rare, chronic, and slowly progressing bacterial infection caused by several members of the *Actinomyces* family. *Actinomyces israelii* is the most common human pathogen among six pathogenic species of *Actinomyces* spp. Although it often causes infection in oral and cervicofacial regions, other regions can also become infected in immunocompromised individuals. PA is mainly caused by aspiration of oropharyngeal or gastrointestinal secretions into the respiratory tract[9].

In cases of endobrochial actinomycosis, bronchiectasis, and chronic obstructive pulmonary disease, corticosteroids, broncholithiasis and endobronchial foreign bodies (e.g., chicken bone and fish bone, grape seed, bean, dental prosthesis, and surgical suture material) are predisposing factors and increase the risk of *Actinomyces* colonization[2,10].

The most common symptoms are fever, weight loss, cough, sputum, chest pain, and hemoptysis. It mimics malignancy or tuberculosis with these nonspecific symptoms and clinical and radiological findings[11].

Pulmonary actinomycosis occurs at any age, but it is more frequent in adults. More frequent infection in men has been

Table 3. Compliance analysis of clinical and radiological diagnoses.

Radiological diagnosis	Clinical diagnosis					
	Malignant	Recurrent malignant	Pneumonia	Infected bronchiectasis	Tuberculosis	Empyema
Malignant	7	0	0	0	0	0
Recurrent malignant	0	1	0	0	0	0
Pneumonia	0	0	5	0	1	1
Infected bronchiectasis	0	0	0	4	0	0
Benign lesions	2	1	0	0	0	0
Total	9	2	5	4	1	1

κ=0.701 and p<0.001.

attributed in part to poor oral hygiene[2,12]. In our study, the majority of the patients were males (n=18, 81.8%).

The main predisposing factors are poor oral hygiene, gingival diseases, surgery, chronic respiratory system disorders such as bronchiectasis, and DM[2]. Among our patients, one patient had a pulmonary cavity and hemoptysis following a tooth extraction, two patients had a history of surgery for lung malignancy causing immunosuppression, and five patients had DM.

The most common clinical symptoms of PA are cough, chest pain, and dyspnea. These nonspecific symptoms make the diagnosis of PA difficult and may often lead to a misdiagnosis of malignancy rather than an infection[9]. Cough, sputum production, and chest pain were reported as the most common complaints in various European studies[13]; however, hemoptysis was more common in Asian series[5].

In our study, the most common complaints of the patients were cough, sputum production, dyspnea, hemoptysis, back chest pain, weight loss, fever, and foul breath, with cough being the most common (n=15) and foul breath being the least common symptom (n=1).

There is no specific and diagnostic imaging modality for PA. Misdiagnosis for malignancy, tuberculosis, or other infections is quite common without a microbiological or histopathological confirmation of the disease[14]. In this study, CXR was normal in 36.8% of the patients, and other findings were nonspecific. The most common site of lesion on thorax CT was the left lower lobe (n=8, 36.4%) and the least frequent site of lesion was the left upper lobe (n=1, 4.5%). The most frequent radiological findings in descending order were mass, cavity, lymphadenopathy (LAP), consolidation, and EBL, comprising 90.9% all radiological findings. As seen, malignancy comes to mind as the first clinical prediagnosis.

In this study, a specimen for histopathologic diagnosis of PA was obtained with rigid bronchoscopy and FOB in 10 patients, and more than one bronchoscopy finding was recorded in the same patient. Considering findings during surgery, stenosis of the bronchus with external compression (n=3, 10%), mucosal irregularity (n=5, 10%), granulation tissue (n=2, 10%), and EBL at the site of the surgical stump (n=2, 10%) suggest lung malignancy as a clinical prediagnosis; however, the presence of the suture material at the site of the surgical stump (n=2, 10%) and aspirated foreign body (a piece of bone) (n=2, 10%) exclude a malignant lesion.

CT and bronchoscopy findings suggesting lung malignancy directed clinicians to order a PET-CT, and 14 (63.6%) patients had PET-CT. SUV_{max} in PET-CT was evaluated according to the cutoff value of SUV_{max}>2.5, which predicts a malignant lesion[15]. There was no pathological uptake on PET-CT in 1 patient, SUV_{max} was <2.5 in 2 patients, and SUV_{max} was >2.5 in 11 (78.5%) patients.

Our patients' PET-CT findings were as follows: collapse consolidation (n=4, 28.6%), EBL (n=3, 21.4%), mass (n=3, 21.4%), nodule (n=2, 14.3%), and cavity (n=2, 14.3%). Mediastinal and hilar LAP was detected in 6 (14%) patients on PET-CT, and the highest SUV_{max} detected in an LAP was 8.52. Actinomycosis, like malignancy, has intense hypermetabolic features and intense FDG uptake; the highest SUV reported in the literature is 33.1[8]. The highest SUV_{max} detected in our patients was 11.77, and the mean SUV_{max} of the lesions was 6.33±3.6.

Since clinical and FOB findings directed the clinician to a clinical diagnosis of malignancy, PET-CT was ordered to support the clinical diagnosis. In this study, it was found that radiological diagnosis was malignancy in 31.8% (n=7) of the patients, and recurrent malignancy in 4.5% (n=1) of them. As a clinical diagnosis, 40.9% (n=9) of our patients were diagnosed with a malignancy, and 9.1% (n=2) of them were diagnosed with a recurrent malignancy. The kappa compliance analysis showed that the clinical and radiological diagnoses were statistically significantly compatible with each other and that the radiological prediagnosis was not superior to the clinical prediagnosis (κ=0.701 and p<0.001).

The kappa compliance analysis performed for all malignancy cases for clinical (n=2) and radiological (n=1) recurrent malignancy diagnoses revealed that the clinical and radiological diagnoses were consistent with each other (κ=0.681 and p<0.001).

It was decided that PET-CT was not useful in terms of predicting malignancy and supporting the clinical diagnosis, and the clinical and radiological diagnoses were found to coincide.

It has been reported that there is a high rate of initial misdiagnosis for malignancy unless PA is confirmed by microbiological or histopathological means[14].

Clinicians should be aware of the predisposing factors of the clinical picture of PA (infection following oral-dental infection, failed tooth extraction, or poor oral-dental hygiene) and that actinomycosis may mimic malignancy in various conditions[10].

There are several limitations in this study: the inclusion of only 22 patients with PA due to the rarity of those cases, the fact that only 14 of the patients had PET-CT, and the lack of inflammatory markers and pulmonary function tests. These may hamper the reliability of the results. Despite these limitations, we believe this study provides important information about the clinical and radiological characteristics of PA.

CONCLUSION

Pulmonary actinomycosis is a rare disorder, the lesion shows high metabolic uptake in PET-CT, and this may mislead clinicians

for a diagnosis of malignancy. Our results suggest that PET-CT does not help distinguish PA from lung malignancy and does not provide a clear diagnostic benefit to the clinician, so the pathological diagnosis is necessary.

THE INSTITUTIONAL BOARD APPROVAL

Health Sciences University Atatürk Chest Diseases and Thoracic Surgery Education and Research Hospital, Medical Education Board approval (decision date February 12, 2019, and number 617).

AUTHORS' CONTRIBUTIONS

HB, FBT, ET: Conceptualization, Data curation, Formal Analysis, Methodology, Writing – original draft. **AFU, DÇ:** Conceptualization, Data curation, Formal Analysis, Methodology, Writing – review and editing.

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ORIGINAL ARTICLE

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Comparison of the effects of duloxetine and pregabalin on pain and associated factors in patients with knee osteoarthritis

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SUMMARY

OBJECTIVES: This study aimed to investigate the effects of duloxetine and pregabalin primarily on pain and functional status in patients with knee osteoarthritis and secondarily on quality of life, depression, anxiety, and sleep disturbance.

METHODS: A total of 66 patients with knee osteoarthritis were randomized to use duloxetine or pregabalin. Patients were evaluated by Visual Analog Scale, Neuropathic Pain Diagnostic Questionnaire, Western Ontario and McMaster University Osteoarthritis Index, Short Form-36, Beck Depression Inventory, Beck Anxiety Inventory, and Pittsburg Sleep Quality Index before the treatment and after 4 and 12 weeks of treatment.

RESULTS: Improvements occurred in Visual Analog Scale, Neuropathic Pain Diagnostic Questionnaire, Western Ontario and McMaster University Osteoarthritis Index, Short Form-36 (with an exception of the mental health subgroup scores in duloxetine-treated group), Beck Depression Inventory, and Beck Anxiety Inventory scores in both groups from 4 weeks after baseline. Pittsburg Sleep Quality Index total scores and SF-36 mental health subgroup scores started to improve on the 4th and 12th weeks in pregabalin- and duloxetine-treated groups, respectively.

CONCLUSION: Osteoarthritis pain, a complex outcome with nociceptive and neuropathic components, leads to central sensitization in a chronic phase. Using centrally acting drugs in the control of pain and associated symptoms would increase the probability of treatment success.

KEYWORDS: Duloxetine hydrochloride. Knee. Osteoarthritis. Pain. Pregabalin.

INTRODUCTION

Osteoarthritis (OA) is a chronic, degenerative disease frequently seen in the middle-aged and elderly people. Knee OA is a particularly common type of OA and a major cause of disability[1]. OA pain is a mixed type of pain involving both nociceptive and neuropathic components. Pain is the most pronounced symptom in knee OA, being initially associated with activity and becoming continuous and severe as the disease progresses. This is thought to be due to the development of central sensitization. Central sensitization is a pain processing abnormality occurring in chronic pain due to persistent activation of the spinal and supraspinal neurons. This process may also cause comorbid conditions such as fear, anxiety, depression, and sleep disorders[2,3]. Central sensitization in knee OA has particularly been shown in patients describing severe pain but without radiological findings indicating the same pain severity[4,5].

Because of the mechanism involved in central sensitization in OA, the use of therapeutic agents that affect the central pain pathways may be required. Duloxetine, a selective serotonin and norepinephrine reuptake inhibitor, is found to be effective in the treatment of chronic pain because of its antidepressant and anxiolytic properties. Duloxetine has been reported to be effective in the treatment of diabetic peripheral neuropathic pain, fibromyalgia, and chronic musculoskeletal pain[6]. Pregabalin suppresses the release of excitatory neurotransmitters by combining with alpha-2-delta subunits of voltage-dependent calcium channels in the central nervous system. It also exhibits positive effects on neuropathic pain-related sleep disturbance, depression, and anxiety. Therapeutic guidelines recommend gabapentinoids (pregabalin-gabapentin) as the first choice medications in the treatment of neuropathic pain[7].

This study was intended to compare the effects of duloxetine and pregabalin on pain and function in patients with OA. The secondary purpose of the study was to examine the effects of these central-acting drugs on anxiety, depression, and sleep disturbance frequently seen during chronic pain and on the

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quality-of-life parameters. To the best of our knowledge, no previous studies have compared the efficacy of pregabalin and duloxetine in knee OA.

METHODS

This prospective, randomized clinical study was performed between October 2016 and December 2020. The study was approved by the Institutional Ethics Committee (FSMEAH-KAEK 9.06.2016/50), and informed consent was obtained from all patients before commencement. Inclusion criteria were age over 40, diagnosis with OA based on the American College of Rheumatology primary knee OA criteria; posterior–anterior X-ray examination showing grades 2–3 knee OA according to the Kellgren and Lawrence Radiological Classification System; a VAS score and a DN4 scale value of 4 or more; and more than 14 painful days a month for at least the past 3 months. Exclusion criteria were body mass index (BMI) higher than 40; the presence of diabetes mellitus, congestive heart failure, cancer, fibromyalgia, inflammatory arthropathy, or autoimmune disease; receipt of invasive treatment with a diagnosis of OA in the past 3 months; being nonambulatory or using assistant devices for walking; and the presence of psychiatric or neurological disease. Notably, 66 patients whose eligibility was confirmed were randomized to one of the two groups, and 3 patients from each group subsequently dropped out from the study; finally, only 60 patients participated in the study. One group was administered 60 mg/day duloxetine HCl, and another group was given 300 mg/day pregabalin, both for 12 weeks. In the duloxetine-treated group, the drug was administered as a single 30 mg dose in the first week and at 60 mg/day from the second week. Patients in the pregabalin-treated group received 75 mg orally twice a day in the first week, followed by 150 mg orally twice a day from the second week. Patient assessments were performed before the treatment and at the 4th and 12th weeks of the treatment. The primary outcome measures were VAS, DN4, and WOMAC, and the secondary outcome measures were BDI, BAI, PSQI, and SF-36. Side effects of the drug were questioned in the patients at the follow-ups.

Statistical evaluation

Data analyses were performed using the Statistical Package for the Social Sciences (SPSS) version 23 software. Frequency and percentage values were calculated for categorical variables, while continuous variables were expressed as mean, standard deviation, median, minimum, and maximum values. Compatibility with a normal distribution of continuous variables was evaluated using the Kolmogorov–Smirnov test. Relationships between categorical variables were determined using the chi-square test. The Mann–Whitney U test was used to compare non-normally distributed variables between two independent groups, and the Friedman test was used for comparisons between more than two groups. Dunn's multiple comparisons test with Bonferroni correction was used for two-way comparisons. Statistical analyses were evaluated at a significance level of 0.05.

RESULTS

No significant difference was determined between the two groups in terms of age, gender, mean duration of disease, Kellgren and Lawrence grade, or initial scores for all the parameters examined. However, BMI was higher in the duloxetine-treated group than that in the pregabalin-treated group (p=0.017). Inter- and intragroup changes in VAS day and night pain, DN4, and WOMAC scores are shown in Table 1. Inter- and intragroup changes in SF-36 and subgroup scores are shown in Table 2. Inter- and intragroup changes in BDI, BAI, and PSQI scores are shown in Table 3.

Side effects were recorded prospectively throughout the study. A total of 16 side effects were observed in the duloxetine-treated group [i.e., constipation (4), dizziness (4), nausea (2), somnolence (1), peripheral edema (1), fatigue (1), pruritus (1), genitourinary symptoms (1), and diarrhea (1)], and 25 side effects were observed in the pregabalin-treated group [i.e., somnolence (6), constipation (4), dizziness (4), weight gain (3), nausea (2), peripheral edema (2), pruritus (1), skin eruption (1), dry mouth (1), and abdominal distension (1)]. In the duloxetine-treated group, three patients were withdrawn from the study (one patient due to pain persisting at the same level of severity, one patient due to constipation, and one patient due to dizziness). In the pregabalin-treated group, 3 patients were dropped out from the study (one patient due to peripheral edema in the bilateral lower extremities and two patients due to somnolence). Other side effects were not severe enough to prevent patients from continuing treatment.

DISCUSSION

In this study, significant improvement was observed compared with pre-treatment values in all parameters in both drug groups from the fourth week. Two exceptions were that total sleep scores and SF-36 MH scores in the duloxetine-treated group only improved compared with baseline in the 12th week. A significant difference between weeks 4 and 12 was also present in a small number of parameters, functional status, and

Table 1. Inter- and intragroup changes in VAS, DN4, and WOMAC scores.

Parameters	Duloxetine (n=30) Mean±SD Median (min–max)	Pregabalin (n=30) Mean±SD Median (min–max)	p*	*Post hoc* test†	Duloxetine	Pregabalin
VAS daytime 0	6.77±1.43 7 [4–10]	7.03±1.54 7 [5–10]	0.476	0 vs. 4	p<0.0001	p<0.0001
VAS daytime 4	3.4±1.92 3 [0–8]	3.4±1.73 3 [1–8]	0.893	0 vs. 12	**p<0.0001**	**p<0.0001**
VAS daytime 12	2.7±2.1 3 [0–8]	2.1±2.12 1 [0–9]	0.179	4 vs. 12	NS	NS
p**	**p<0.0001**	**p<0.0001**				
VAS nighttime 0	6.83±1.7 7 [4–10]	6.73±2.53 8 [0–9]	0.647	0 vs. 4	**p<0.0001**	**p<0.0001**
VAS nighttime 4	3.57±1.96 3.5 [0–8]	2.97±1.94 3 [0–8]	0.242	0 vs. 12	**p<0.0001**	**p<0.0001**
VAS nighttime 12	2.6±2.14 2.5 [0–8]	1.9±2.02 2 [0–9]	0.152	4 vs. 12	NS	NS
p**	**p<0.0001**	**p<0.0001**				
DN4-0	5.27±1.6 5 [4–9]	4.9±1.21 4 [4–8]	0.410	0 vs. 4	**p<0.0001**	**p<0.0001**
DN4-4	3.03±1.63 3 [0–7]	1.47±1.2 1 [0–5]	**p<0.0001**	0 vs. 12	**p<0.0001**	**p<0.0001**
DN4-12	2.73±1.62 3 [0–7]	1.43±1.28 1 [0–5]	**0.001**	4 vs. 12	NS	NS
p**	**p<0.0001**	**p<0.0001**				
WOMAC 0 pain	13.37±3.83	12.47±4.36	0.377	0 vs. 4	**p<0.0001**	**p<0.0001**
WOMAC 4 pain	8.67±4.36	6.53±3.72	**0.038**	0 vs. 12	**p<0.0001**	**p<0.0001**
WOMAC 12 pain	7.2±5.61	4.2±4.29	**0.020**	4 vs. 12	NS	**0.024**
p**	**p<0.0001**	**p<0.0001**				
WOMAC 0 stiffness	5±2.27	4.03±2.16	0.101	0 vs. 4	**p<0.0001**	**p<0.0001**
WOMAC 4 stiffness	3.33±2.2	2±1.82	**0.016**	0 vs. 12	**p<0.0001**	**0.001**
WOMAC 12 stiffness	3±2.17	1.37±1.69	**0.002**	4 vs. 12	NS	NS
p**	**p<0.0001**	**p<0.0001**				
WOMAC 0 function	40.6±14.66	38.1±14.56	0.662	0 vs. 4	**p<0.0001**	**p<0.0001**
WOMAC 4 function	26.2±13.58	21.03±14.3	0.141	0 vs. 12	**p<0.0001**	**p<0.0001**
WOMAC 12 function	22.93±17.08	14.5±14.98	**0.043**	4 vs. 12	NS	NS
p**	**p<0.0001**	**p<0.0001**				
WOMAC 0 total	60.62±19.17	56.52±20.3	0.473	0 vs. 4	**p<0.0001**	**p<0.0001**
WOMAC 4 total	39.3±19.47	30.9±19.59	0.109	0 vs. 12	**p<0.0001**	**p<0.0001**
WOMAC 12 total	34.02±23.81	20.9±21.28	**0.022**	4 vs. 12	NS	**0.029**
p**	**p<0.0001**	**p<0.0001**				

*Mann–Whitney U test; **Friedman test. †Dunn's multiple comparisons test with Bonferroni correction. **Min:** minimum; **Max:** maximum; **SD:** standard deviation; **VAS:** Visual Analog Scale; **NS:** nonsignificant. Bold indicates statistically significant values.

quality-of-life subscores. A comparison of duloxetine and pregabalin treatment revealed that pregabalin was superior in terms of its effects on the neuropathic component of pain, functional status, and some quality-of-life parameters.

Earlier, OA pain was generally regarded as a peripherally mediated nociceptive pain. However, inflammatory mediators have been shown to modulate both peripheral and central nociceptors following intra-articular release from damaged tissues[8].

Table 2. Inter- and intragroup SF-36 and sub-parameter changes.

Parameters	Duloxetine (n=30) Mean±SD	Pregabalin (n=30) Mean±SD	p*	*Post hoc* test†	Duloxetine	Pregabalin
SF-36 PF 0	43.17±17.79	41.67±21.15	0.917	0 vs. 4	**p=0.001**	**p=0.001**
SF-36 PF 4	64±22.22	64±20.98	0.870	0 vs. 12	**p<0.0001**	**p<0.0001**
SF-36 PF 12	71.08±21.69	76±22.61	0.237	4 vs. 12	NS	**0.035**
p**	**p<0.0001**	**p<0.0001**				
SF-36 PRF 0	20.22±35.97	20.83±34.79	0.844	0 vs. 4	**0.002**	**0.002**
SF-36 PRF 4	62.21±40.75	69.17±44.37	0.414	0 vs. 12	**p<0.0001**	**p<0.0001**
SF-36 PRF 12	68.44±39.31	83.33±33.69	0.112	4 vs. 12	NS	NS
p**	**p<0.0001**	**p<0.0001**				
SF-36 ERF 0	38.87±35.1	31.09±28.93	0.450	0 vs. 4	**p<0.0001**	**p<0.0001**
SF-36 ERF 4	69.96±25.31	71.1±37.89	0.463	0 vs. 12	**0.007**	**0.011**
SF-36 ERF 12	80.53±26.31	85.55±29.93	0.184	4 vs. 12	NS	NS
p**	**p<0.0001**	**p<0.0001**				
SF-36 V 0	40.11±22.39	36.73±24.34	0.276	0 vs. 4	**p<0.0001**	**p<0.0001**
SF-36 V 4	48±22.54	55.33±22.7	0.194	0 vs. 12	**0.014**	**0.014**
SF-36 V 12	58.27±21.55	64±18.82	0.357	4 vs. 12	**0.024**	NS
p**	**p<0.0001**	**p<0.0001**				
SF-36 MH0	49.5±23.49	50.8±24.14	0.716	0 vs. 4	NS	**p<0.0001**
SF-36 MH 4	55.83±17.53	66.4±18.98	**0.014**	0 vs. 12	**0.006**	**0.002**
SF-36 MH 12	61.23±21.4	71.67±14.73	0.055	4 vs. 12	NS	NS
p**	**p<0.0001**	**p<0.0001**				
SF-36SRF 0	56.85±24.19	49.58±26.16	0.187	0 vs. 4	**0.020**	**0.001**
SF-36 SRF 4	69.5±25.15	72.92±21.3	0.799	0 vs. 12	**p<0.0001**	**p<0.0001**
SF-36 SRF 12	75.03±22.12	78.75±17.1	0.689	4 vs. 12	NS	NS
p**	**p<0.0001**	**p<0.0001**				
SF-36 BP 0	31.33±18.47	23.17±17.37	0.061	0 vs. 4	**p<0.0001**	**p<0.0001**
SF-36 BP 4	58.42±24.05	62.25±21.79	0.336	0 vs. 12	**p<0.0001**	**p<0.0001**
SF-36 BP 12	63.75±29.41	66.83±25.02	0.911	4 vs. 12	NS	NS
p**	**p<0.0001**	**p<0.0001**				
SF-36 GHP 0	36.5±20.14	42.1±23.47	0.509	0 vs. 4	**p<0.0001**	**0.002**
SF-36 GHP 4	50.23±16.62	59.57±18.02	0.063	0 vs. 12	**p<0.0001**	**p<0.0001**
SF-36 GHP 12	56.92±20.59	69.07±14.62	**0.022**	4 vs. 12	NS	NS
p**	**p<0.0001**	**p<0.0001**				

*Mann–Whitney U test; **Friedman test. †Dunn's multiple comparisons test with Bonferroni correction. Bold indicates statistically significant values. SF-36: Short Form-36; PF: physical functioning; PRF: physical role functioning; ERF: emotional role functioning; V: vitality; MH: mental health; SRF: social role functioning; BP: bodily pain; GHP: general health perceptions; SD: standard deviation; VAS: Visual Analog Scale; NS: nonsignificant.

Patients with OA are now known to experience varying degrees of both nociceptive and neuropathic pain[9]. Significant scientific evidence also reports a role for central sensitization in OA pain. The presence of central sensitization confuses the clinical picture and makes it less likely to respond to conventional treatments[10]. The addition of centrally acting agents to conventional therapies in the treatment of OA has been shown to increase the response to treatment[11].

Table 3. Inter- and intragroup changes in BDI, BAI, and Pittsburg scores.

Parameters	Duloxetine (n=30) Mean+SD	Pregabalin (n=30) Mean+SD	p*	*Post hoc* test†	Duloxetine	Pregabalin
BDI 0	17.2±8.72	16.53±9.21	0.841	0 vs. 4	**p<0.001**	**p<0.017**
BDI 4	11.63±7.65	10.47±8.11	0.501	0 vs. 12	**p<0.0001**	**p<0.0001**
BDI 12	10.13±7.88	7.83±6.26	0.310	4 vs. 12	NS	NS
p**	**p<0.0001**	**p<0.0001**				
BAI 0	17.63±13.63	17.63±9.98	0.594	0 vs. 4	**0.002**	**0.001**
BAI 4	13.07±11.23	10.87±7.59	0.706	0 vs. 12	**p<0.0001**	**p<0.0001**
BAI 12	11±9.67	7.3±6.18	0.109	4 vs. 12	NS	NS
p**	**p<0.0001**	**p<0.0001**				
Pittsburg total-0	8.93±4.87	11±4.99	0,119	0 vs. 4	NS	**0.001**
Pittsburg total-4	7.13±5.11	6.93±4.37	0.795	0 vs. 12	**0.001**	**p<0.0001**
Pittsburg total-12	6.27±5.09	5.9±3.52	0.522	4 vs. 12	NS	NS
p**	**p<0.0001**	**p<0.0001**				

*Mann–Whitney U test; **Friedman test. †Dunn's multiple comparisons test with Bonferroni correction. Bold indicates statistically significant values. BAI: Beck Anxiety Inventory; BDI: Beck Depression Inventory; NS: nonsignificant.

There have been very few studies of the use of pregabalin in knee OA. Rahman et al. described pregabalin as useful against pain resulting from knee OA in an animal model and demonstrated modified physiology of deep dorsal horn wide dynamic range neurons, suggesting an association between heightened neuronal activity and hypersensitive behavioral responses[12]. In another study using a lower pregabalin dosage than in this study, patients with knee OA were randomized into groups receiving meloxicam 10 mg, meloxicam 10 mg/day+pregabalin 25 mg/day, and pregabalin 25 mg/day only. Significant improvements were recorded in the meloxicam+pregabalin group in terms of pain and functional status scores, showing that knee OA represents a combination of both nociceptive and neuropathic pain[11]. In another study, the same number of patients as in this study were randomized into aceclofenac- and aceclofenac+pregabalin-treated groups, and significant improvement in both pain severity and functional status was observed in the combination therapy group compared with the monotherapy group[13].

There have been more studies investigating the use of duloxetine in knee OA than pregabalin. A meta-analysis of three randomized controlled studies involving a total of 1011 patients reported significant improvement in pain and functional status in patients using 60/120 mg duloxetine following approximately 10–13 weeks of treatment compared with a placebo group. Similar to this study, tolerable levels of side effects such as nausea, fatigue, constipation, hyperhidrosis, somnolence, dizziness, diarrhea, insomnia, and dry mouth were reported in the three studies in that meta-analysis[14]. A double-blinded randomized, controlled study of 354 patients reported no change in knee joint movement and x-ray findings in a duloxetine-treated group but observed significant improvement in pain and functional status compared with a control group[6]. Another study of 288 patients with knee OA aged over 65 reported significant improvements in pain, functionality, and geriatric depressions scores in a duloxetine-treated group compared with a placebo group[15]. A study comparing the efficacy of pregabalin and duloxetine in patients with hand OA reported that both agents were effective against chronic hand OA pain, with pregabalin being superior to duloxetine. However, in contrast to the findings of this study, neither of the two drugs was reported to produce any improvement in either depression or anxiety scores[16].

Chronic pain developing in association with OA can result in impairment of health-related quality of life and daily activities, and psychological distress, including depression[17] and sleep disturbances[18]. The prevalence of depression is estimated to be two to three times higher in patients with OA[19-20]. Sarıyıldız et al. reported impairment of sleep quality in patients with knee OA and that this was particularly associated with knee pain, age, depressive symptoms, and radiological grade[21]. Alkan et al. reported lower SF-36 scores among patients with OA compared with healthy controls[22]. In this study, treatment with both duloxetine and pregabalin improved anxiety, depression, and sleep disturbance symptoms, together with the quality of

life. Despite not reaching statistical significance, pregabalin was superior to duloxetine in all these parameters, and pregabalin also exhibited its effect on sleep disturbance earlier. Studies examining the effectiveness of pregabalin on anxiety and depression have reported inconsistent findings regarding pain, but similar results in terms of its effect on sleep. Gilron et al. reported that pregabalin yielded a small but significant difference in sleep interference, anxiety, and depression scores[23]. This study has some limitations. The first limitation is the absence of a control group. Although we knew that using a placebo group would make our study more valuable, we did not use a placebo group because we thought that the quality of life of the patients might be affected by this process. The second one is the short follow-up period, since more informative results might have been yielded by a longer follow-up period. The third limitation is that BMI was higher in the duloxetine group than that in the pregabalin group. This may partially account for the superiority of pregabalin in terms of knee functionality.

CONCLUSION

Pain in OA is a complex event involving both nociceptive and neuropathic components. According to the results of our study, both duloxetine and pregabalin are effective in reducing mixed type of pain and improving functions. These agents also are useful against depression, anxiety, and sleep disorder, which frequently accompany the chronic process, and can thus contribute to an improvement of the patient's quality of life.

ETHICAL APPROVAL

The study was approved by the Institutional Ethics Committee (FSMEAH-KAEK 9.06.2016/50). Clinical trial ID: NCT04532684, retrospectively registered 25.01.2021.

AUTHORS' CONTRIBUTIONS

OGI: Contributed to the conceptualization, data curation, formal analysis, investigation, methodology, and writing – original draft. **KNKO:** Contributed to the data curation, investigation, methodology, validation, and visualization. **IA:** Contributed to the project administration, methodology, supervision and writing – review & editing. **FUO:** Contributed to the methodology, supervision, and writing – review & editing. **TN:** Contributed to the data curation, investigation, and resources. **FAB:** Contributed to the data curation, investigation, and resources. **MYK:** Contributed to the data curation, investigation, and resources. **AA:** Contributed to the data curation, investigation, and resources. **PA:** Contributed to the data curation, investigation, and resources.

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ORIGINAL ARTICLE

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The prognostic value of the CHA_2DS_2-VASc score in coronary collateral circulation and long-term mortality in coronary artery disease

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SUMMARY

BACKGROUND: The CHA_2DS_2-VASc score is used to determine thromboembolic risk in cases of atrial fibrillation. The predictive value of this score in predicting coronary collateral circulation in chronic total occlusion is unknown.

OBJECTIVE: The aim of this study was to investigate the relationship between the CHA_2DS_2-VASc score and coronary collateral circulation in patients with chronic total occlusion.

METHODS: A total of 189 patients, who underwent coronary angiography and had a chronic total occlusion in at least one coronary artery, were enrolled in this study. The Rentrop scoring system was used for grouping the patients, and patients were classified as having poorly developed coronary collateral circulation (Rentrop grade 0 or 1) or well-developed coronary collateral circulation (Rentrop grade 2 or 3).

RESULTS: The CHA_2DS_2-VASc score of the good coronary collateral circulation group was significantly lower than the other group (3.1±1.7 vs. 3.7±1.7, p=0.021). During the follow-up period, 30 (32.2%) patients in the poorly developed coronary collateral circulation group and 16 (16.7%) patients in the well-developed coronary collateral circulation group died (p=0.028). According to the multivariable Cox regression model, the CHA_2DS_2-VASc score [hazard ratio (HR): 1.262, p=0.009], heart rate (HR: 1.049, p=0.003), LVEF (HR: 0.975, p=0.039), mean platelet volume (HR: 1.414, p=0.028), and not taking acetylsalicylic acid during admission (HR: 0.514, p=0.042) were independently associated with a higher risk of mortality.

CONCLUSIONS: The CHA_2DS_2-VASc score is closely related to coronary collateral development and predicts mortality in patients with chronic total occlusion.

KEYWORDS: Collateral circulation. CHA_2DS_2-VASc score. Rentrop grade. Coronary occlusion. Coronary artery disease. Mortality.

INTRODUCTION

Coronary collateral circulation (CCC) is a physiologic protective mechanism of a heart in case of critical coronary artery stenosis and ischemia. A well-developed CCC reduces myocardial ischemia and anginal symptoms, protects cardiac function, improves the prognosis of patients with stable coronary artery disease (SCAD), and reduces the incidence of cardiogenic shock and mortality after acute myocardial infarction (MI)[1]. However, the influencing factors and possible mechanisms of collateral circulation formation in patients with CAD have not been fully elucidated. In previous studies, inflammation and inflammatory markers such as C-reactive protein (CRP) and acute-phase reactants were found to be responsible for poor CCC development[2].

The CHA_2DS_2-VASc (congestive heart failure, hypertension, age ≥75 years [doubled], diabetes mellitus, previous stroke or transient ischemic attack (TIA) [doubled], vascular disease, age 65–74 years, female gender) score was first applied to patients with atrial fibrillation (AF) to determine their thromboembolic risks. Recently, some studies have found a positive correlation between CHA_2DS_2-VASc score and increased inflammatory status[3,4].

However, the association between CHA_2DS_2-VASc score and CCC has not been investigated. Our aim in this study was to determine the relationship between CHA_2DS_2-VASc score and CCC and mortality in patients with CAD.

METHODS

A total of 189 patients, who underwent coronary angiography (CAG) and had a chronic total occlusion (CTO) in at least one coronary artery between January 2019 and January 2021, were enrolled in this study. CTO was defined as a total occlusion of a coronary vessel with a distally TIMI 0 or TIMI 1 flow for at least 3 months. Patients were excluded from the study if they had acute coronary syndrome in the

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past 3 months, coronary artery bypass surgery of coronary stenting before, symptomatic heart failure (NHYA class III or IV), severe cardiac valvular disease (class III or IV), hepatic failure, active infectious disease, malignancy, and thyroid disease. The study protocol was approved by the local ethics committee, and the study was conducted in accordance with the Declaration of Helsinki.

Basic demographic information regarding age and sex, and CAD risk factors (e.g., hypertension, diabetes, dyslipidemia, history of smoking, and family history) were obtained from the hospital database. Blood results taken before CAG were screened from hospital records, and fasting blood glucose and cholesterol parameters were determined. Left ventricular ejection fraction (LVEF) was also gathered from echocardiography records, which was done before or after CAG. Medications of the patients were recorded. Patients who were taking antihypertensive medications were categorized as hypertensives. Dyslipidemia was defined according to the European Society of Cardiology guidelines[5]. Diabetics were determined by patients who had already been diagnosed with diabetes and taking antidiabetic medications and other patients who did not know their diabetes status but had high blood glucose according to the American Diabetes Association's criteria[6].

Coronary angiography was performed by transfemoral or transradial access with the routine Judkins technique. CAG data were interpreted by two cardiologists, and CCC was classified by the Cohen-Rentrop standard[7]. According to Rentrop classification, grade 0 was defined as no visible collateral circulation, grade 1 was defined as filling of the side branches without distal main epicardial vessel filling, and grades 2 and 3 were defined as partial and complete filling of the distal main epicardial vessel, respectively. Patients who had grades 0 and 1 were accepted as having poor collaterals, whereas patients who had grades 2 and 3 were accepted as having good collaterals.

The CHA_2DS_2-VASc score was calculated according to the following criteria: 1 point for the presence of chronic heart failure (LVEF<40%), hypertension, diabetes, age between 65 and 74, vascular disease (previous MI or CAD and peripheral arterial disease), and female sex; and 2 points for the presence of stroke and/or TIA history and age 75 or above. Patients get a CHA_2DS_2-VASc score between 0 and 9.

Analysis of statistics was performed by using SPSS version 22.0 Statistical Package Program for Windows (SPSS Inc., Chicago, IL, United States). The distribution pattern of parameters, whether normal or not, was determined by the Kolmogorov–Smirnov test. Continuous parameters with normal distribution were presented as mean±standard deviation, whereas parameters with non-normal distribution were presented as median (interquartile range); categorical variables were presented with number and percentage values. A Student's t-test was used to compare continuous variables between groups when the distribution was normal, and the rank-sum test (Mann–Whitney U-test) was used to compare continuous variables between groups when the distribution was non-normal. The chi-square test was used to compare categorical variables. The effects of different variables on mortality were assessed by the Cox regression analysis. Inclusion of covariates in the multivariable model was first determined by selecting those that exhibited two-sided $p<0.10$ in unadjusted analyses. Inclusion of additional covariates was determined by performing a stepwise backward selection process until all the other variables in the model exhibited $p<0.10$. The survival curves during hospitalization for the collateral groups were analyzed using the Kaplan–Meier method, and statistical assessment was performed using the log-rank test. A p-value of <0.05 was considered statistically significant.

RESULTS

A total of 189 patients were divided into two groups according to the Rentrop grades. The poorly developed CCC group (Rentrop 0 or 1) comprised of 93 patients, and 96 patients had well-developed CCC (Rentrop 2 or 3). Both groups were similar in age, sex, presence of diabetes, hypertension, hyperlipidemia, and smoking status. There was no significant difference in terms of heart rate (HR), systolic blood pressure, diastolic pressure, and previous medications other than acetylsalicylic acid (ASA). However, the LVEF of the poor collateral group was significantly lower (p=0.033).

The CHA_2DS_2-VASc score of the well-developed collateral group was significantly lower than the other group (3.1±1.7 vs. 3.7±1.7, p=0.021). The mean duration of the follow-up was 31.5±13.9 months. During the follow-up period, 30 (32.2%) patients in the poorly developed CCC group and 16 (16.7%) patients in the well-developed CCC group died (p=0.028). When we grouped the patients according to the CHA_2DS_2-VASc score <2 and ³2, the mortality rate was also significantly higher in CHA_2DS_2-VASc score group (31% vs. 7%, p<0.001) (Table 1). Hemoglobin and platelet counts, glomerular filtration rate, and cholesterol levels have no significant difference in the two groups.

In the multivariable Cox regression model, the CHA_2DS_2-VASc score [hazard ratio (HR): 1.262, p=0.009], HR (1.049, p=0.003), LVEF (HR: 0.975, p=0.039), mean platelet

Table 1. Demographic and clinical characteristics of the study population according to CHA_2DS_2-VASc score.

Characteristics	All patients (n=189)	CHA_2DS_2-VASc≤2 (n=55)	CHA_2DS_2-VASc>2 (n=134)	p-value
Age (years), mean±SD	68.2±10.7	61±8.3	71.2±10.1	**<0.001**
Male, n (%)	154 (81.4)	53 (96.4)	101 (75.3)	**<0.001**
SBP, mm Hg	127.6±17.8	124.9±15	128.7±18.9	0.178
DBP, mm Hg	75.6±12.3	73.4±9.2	76.6±13.3	0.109
Current smoker, n (%)	40 (21.1)	20 (36.4)	20 (14.9)	**0.002**
CKD, n (%)	52 (27.5)	6 (10.9)	46 (34.3)	**0.001**
Hyperlipidemia, n (%)	17 (8.9)	4 (7.3)	13 (9.7)	0.596
Family history of CVD, n (%)	16 (8.5)	4 (7.3)	12 (8.9)	0.706
Prior MI, n (%)	87 (46)	10 (18.1)	77 (57.4)	**<0.001**
Multivessel disease, n (%)	134 (70.9)	39 (70.9)	95 (70.9)	1
EF, %, mean±SD	46.3±12.4	51.7±11.2	44.1±12.2	**<0.001**
ECG, n (%)				
Sinus rhythm	175 (92.6)	54 (98.2)	121 (90.3)	0.060
Atrial fibrillation	14 (7.4)	1 (1.8)	13 (9.7)	0.060
CTO location, n (%)				
LAD	50 (26.4)	15 (27.2)	35 (26.1)	0.858
Cx	28 (14.8)	11 (20)	17 (12.7)	0.259
RCA	104 (55)	26 (47.3)	78 (58.2)	0.199
Other	25 (13.2)	6 (10.9)	19 (14.2)	0.642
Gensini score, mean±SD	59.8±25.2	62.2±24.8	58.9±25.4	0.406
CCC, n (%)				
Poorly developed	93 (49.2)	19 (34.5)	77 (57.5)	**0.006**
Well developed	96 (50.8)	36 (65.4)	57 (42.5)	**0.006**
CHA_2DS_2-VASc score, mean±SD	3.4±1.7	1.3±0.7	4.2±1.2	**<0.001**
Mortality, n (%)	46 (24.3)	4 (7.3)	42 (31.3)	**<0.001**

CCC: coronary collateral circulation; CHF: chronic heart failure; CKD: chronic kidney disease; CTO: chronic total occlusion; CVD: cardiovascular disease; Cx: circumflex coronary artery; DBP: diastolic blood pressure; EF: ejection fraction; LAD: left anterior descending coronary artery; LDL-C: low-density lipoprotein cholesterol; RCA: right coronary artery; SBP: systolic blood pressure. Bold indicates statistically significant values.

volume (MPV) (HR: 1.414, p=0.028), and not taking ASA during admission (HR: 0.514, p=0.042) were independently associated with a higher risk of mortality (Table 2). Finally, Kaplan–Meier survival curves stratified by the CHA_2DS_2-VASc score (≤2 vs. >2) and CCC groups (well vs. poor) represented that higher CHA_2DS_2-VASc score and poor CCC were associated with higher mortality rates, as shown in Figure 1.

DISCUSSION

To the best of our knowledge, this is the first study that shows the CHA_2DS_2-VASc score is related to coronary collateral development and predicts long-term mortality in patients with CAD. Other than the CHA_2DS_2-VASc score, increased HR, low LVEF, increased platelet volume, and not taking ASA during admission were also found to be related to mortality.

Collaterals are the small vessel formations that supply blood to the ischemic area[8]. It works as a compensatory mechanism to protect myocardial muscles from ischemic damage in case of obstruction of the main epicardial vessels. Studies have shown that patients with good collaterals have a better myocardial contractile function, lower infarct size, and positive remodeling process after acute MI[1]. However, although the severity of the obstruction of the epicardial vessels is the same, some patients have well-developed collaterals but some patients do

not. Several studies have been conducted to clarify this issue until now, and it has been clearly demonstrated that higher inflammatory status in the myocardium and higher inflammatory markers are responsible for the prevention of the development of collaterals[9,10].

The CHA_2DS_2-VASc score is a well-validated clinical scoring system to predict thromboembolic risk in patients with non-valvular AF[11]. In previous studies, this score has been found to be related to increased inflammation and worse prognosis in patients with many diseases like CAD[12], heart failure[13], stroke[14], chronic kidney disease[15], and, recently, COVID-19[16]. We found that higher CHA_2DS_2-VASc score causes low-grade collateral formation in coronary vessels. This result can be interpreted with the negative effects of inflammation on collateral formation, because the components of the CHA_2DS_2-VASc score are the well-known risk factors for atherosclerosis, which is a chronic inflammatory process of the coronary vessel.

Another important result of our study is the increased mortality rates of the poorly developed collateral group during follow-up, and this can be the consequence of both the high CHA_2DS_2-VASc score and the presence of poor collaterals. Because both of these scenarios have been shown as the cause of increased mortality in CAD patients earlier[12,17]. In a meta-analysis that included about 6500 patients earlier, it was shown that patients with high collateralization had a 36% reduced mortality risk compared with patients with low collateralization[17]. However, in our study according to the Cox regression analysis, although the CHA_2DS_2-VASc score increases mortality risk with a HR of 1.262, coronary collateral grades were not found to be related to mortality. This may be the effect of the presence of robust predictors like HR, ejection fraction, MPV, red cell distribution width, and ASA usage in the regression model.

There are some limitations to this study. First, this is a cross-sectional, single-center, retrospective study, which has a relatively small sample size; our study cannot clarify the exact mechanisms linking with the CHA_2DS_2-VASc score and poor development of collaterals in patients with CAD, and thus, further studies are needed. Second, the CCC classification adopts the Rentrop classification standard, which means that the small, microscopic vessels may not be visualized angiographically.

Table 2. Multivariable Cox regression analysis of risk factors for mortality.

Variables*,**	Hazard ratio, 95%CI	p-value
Hemoglobin	0.862 (0.741–1.002)	0.053
Mean platelet volume	1.414 (1.039–1.925)	**0.028**
Heart rate	1.020 (1.001–1.039)	**0.038**
Acetylsalicylic-acid usage	0.514 (0.271–0.977)	**0.042**
Ejection fraction	0.975 (0.952–0.999)	**0.039**
Gensini score	1.010 (1.000–1.020)	0.059
CHA_2DS_2-VASc score	1.262 (1.060–1.502)	0.009

CI: confidence interval. Bold indicates statistically significant values. *Model included age, diabetes, stroke, heart rate, ejection fraction, hemoglobin, mean platelet volume, red cell distribution width, acetylsalicylic acid usage, presence of chronic total obstruction in the right coronary artery, the circumflex artery, and the left anterior descending artery, collateral grade, Gensini score, and CHA_2DS_2-VASc score. **Selection of covariates for multivariable models is explained in the "Methods" section. Unless otherwise indicated, hazard is interpreted as the presence (vs. absence) of each categorical variable or an increase of 1 unit of each continuous variable.

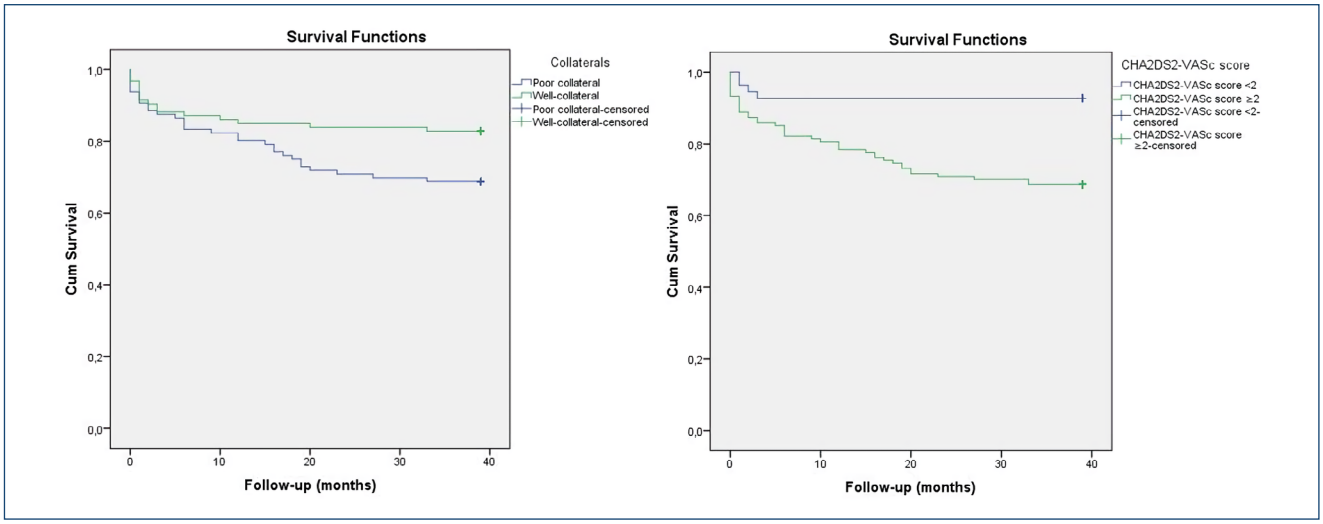

Figure 1. Kaplan–Meier survival curves stratified by the CHA_2DS_2-VASc score and coronary collateral circulation for mortality.

CONCLUSION

In this study, we have found a relationship between the CHA_2DS_2-VASc score, which has an expanding clinical use, and collateral development and mortality. This is an easy score to calculate and may be very useful to find high-risk patients with poor CCC for adverse cardiac events and mortality.

AUTHORS' CONTRIBUTIONS

MKA: contributed to the conception and study design, acquisition, analysis, interpretation of data, and manuscript revision. **AT:** contributed to the acquisition, analysis, and interpretation of data. **SY:** contributed to the conception and study design, acquisition, analysis, interpretation of data, and manuscript revision.

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ORIGINAL ARTICLE

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An examınatıon of the anxıety states of Turkısh health care workers durıng the COVID-19 pandemıc: a cross-sectıonal study

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SUMMARY

OBJECTIVE: The aim of this study was to evaluate the anxiety experienced by health care workers in different branches during the COVID-19 pandemic.
METHODS: The cross-sectional study included 373 health care workers. Data were collected using an online questionnaire consisting of the Sociodemographic Form (32 items related to the working conditions of health care professionals during the COVID-19 pandemic) and the Penn State Concern Questionnaire.
RESULTS: The anxiety levels of the female workers were significantly higher (p<0.001). The total Penn State Concern Questionnaire points were determined to be statistically significantly higher in those who need to protect the family during the pandemic (p=0.03), who were dissatisfied with their profession (p<0.001), and those whose workload had increased during the pandemic (p=0.007).
CONCLUSIONS: The study results demonstrated that the levels of anxiety of health care workers during the COVID-19 pandemic could be increased by young age, low level of experience, female gender, increased workload, and dissatisfaction with the profession.
KEYWORDS: Healthcare workers. Anxiety. COVID-19 pandemic. SARS-CoV-2.

INTRODUCTION

The field of health care is a professional arm that brings different professional groups together to provide health care services under intense and hard working conditions[1]. Health care professionals have the responsibility and an important role in combatting the COVID-19 pandemic, and they are a high-risk group regarding infection[2]. They may experience various health problems while delivering health care services. The intense stress felt by patients and their relatives because of uncertainties and unknown aspects of the disease can have a negative impact on health care personnel[3]. This situation experienced by health care professionals reveals the concept of anxiety, which is difficult to control[4-6].

The emergence of potentially negative working conditions in the field of health care can lead to quantitative and qualitative deteriorations in the work conducted[7,8]. It is thought that negative situations, such as intense work tempo, epidemics, anxiety about becoming infected and spreading disease to family, and the need to support patients and their relatives, affect work-related tension and stress[9]. These situations can have a negative effect on work performance and lead to outcomes such as decreased job satisfaction or leaving work[10]. In a recent study, it was reported that health care workers who were not sure whether or not they were infected with COVID-19 were more anxious and worried, and less satisfied with their job, and that those in the private sector had better mental health than those in the public sector[11].

The COVID-19 pandemic has deeply affected the whole world, and to be able to better explain the effects and to determine effective treatment methods, scientific studies have been and are still being conducted[12,13]. Studies that have evaluated the effects of COVID-19 on health care workers have generally focused on health care professionals working in certain countries. Most studies examined the anxiety status of health care professionals working in China. Considering that different countries have different health policies, the pandemic effects on health care professionals in other countries are needed to be investigated[14]. The aim of this study was to evaluate the anxiety experienced by Turkish health care workers who are making great efforts in the struggle against the COVID-19 pandemic.

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METHODS

Design and study participants

This cross-sectional study included health care personnel who actively work in public or private health care institutions. The sample size was calculated using the G.Power-3.1.9.2 software. As a result of the analysis, it was determined that a sample size of 373 subjects provided an effect size of 0.3636 at α=0.05, and the study power calculated *post hoc* after the study was 0.91. The minimum power value required for the *post hoc* analysis was found to be 0.67. Therefore, the sample size was at an acceptable level.

Inclusion criteria were as follows: health care personnel with active duties in public or private health care institutions during the COVID-19 pandemic, no communication disability, and no physical disability. Employees other than health care personnel (e.g., imam, security staff, and cleaning staff) and those who did not want to participate were excluded from the study.

Data collection tools

Data were collected with an online questionnaire created on the Internet. This online questionnaire consisted of two sections of the Sociodemographic Form (32 items related to the working conditions of the health care personnel during the COVID-19 pandemic) and the Penn State Worry Questionnaire (PSWQ). The online questionnaire was created using Google Forms and was shared on different Internet platforms (e.g., WhatsApp, Gmail, Facebook, Instagram, and Twitter). The study data were shared by the researchers at specific intervals (2 days a week) between August and October 2020, and the responses were recorded on the Internet.

Penn State Worry Questionnaire

The Turkish version of PSWQ was used to assess the participants' anxiety levels. It is a self-reported scale comprising 16 items with 5-point Likert-type responses, score ranging from 1 (this is never true for me) to 5 (this is always true for me). Higher points indicate a higher level of anxiety[15].

Statistical analysis

Data obtained in the study were analyzed statistically using SPSS version 25 software (Statistical Package for Social Sciences). The conformity of the research data set to normal distribution was assessed with the Shapiro-Wilk test. When evaluating the descriptive statistics of the study participants, number (n) and percentage (%) distribution and mean±standard deviation (SD) values were used. The independent samples t-test was used for the comparisons of two groups of numerical data, and for more than two groups of independent variables, one-way ANOVA was used. Multiple linear regression analysis was used to determine the change in anxiety. A dummy variable was used in the model. The reliability of the scales used in the study was examined using the Cronbach's alpha reliability coefficient. A value of $p<0.05$ was accepted as statistically significant. Permission to conduct the study was obtained from the IU-C Research Ethics Committee (Decision No: 60116787-020/41133).

RESULTS

The research was completed with the data of 373 health care professionals who met the study criteria and agreed to participate in the study. The mean age of the study subjects was 34.42±10.52 years (range: 21–73 years), and the mean duration of professional experience was 11.35±10.23 years (range: 1–45 years). When the PSWQ points were compared according to gender, a statistically significant difference was determined regarding females ($p<0.001$) (Table 1).

In the comparison of the PSWQ total scores according to the responses to questions, a statistically significant difference was determined in the anxiety scores of feeling the need to protect the family during the pandemic ($p=0.03$), infection of someone in their close environment ($p=0.04$), being dissatisfied with the job ($p<0.001$), worrying about becoming infected ($p<0.001$), thinking that COVID-19 precautions are insufficient ($p<0.001$), being exposed to heavy workload ($p=0.007$), and wishing to change profession ($p<0.001$) (Table 2).

According to the regression analysis results, female workers (β: 0.132, $p=0.009$), those with somebody infected in their close environment (β: 0.104, $p=0.043$), those who feared becoming infected (β: 0.202, $p<0.001$), and those who wished to change their profession (β: 0.109, $p=0.047$) were found to be more worried. The change occurring in the scale total was found to be explained by independent variables at the rate of 13% (R^2=0.130). As the Durbin and Watson value was between 1.5 and 2.5, this showed that there was no autocorrelation problem in the model (Durbin-Watson=2.363) (Table 3).

DISCUSSION

According to the results of this study, which examined the effect of the COVID-19 pandemic on the anxiety state of health care workers, lower age and professional experience were found to increase the level of anxiety. Female health care workers, those who felt the need to protect their family during

Table 1. Comparison of the participants' Penn State Anxiety Total Scores according to the descriptive characteristics.

Variables		n	%	X	SD	Test value	p
Age (years)		373		34.42	10.52		
Professional experience (years)		373		11.35	10.23		
Gender	Male	132	35.4	45.54	11.51	-3.249**	**0.001***
	Female	241	64.6	49.80	12.43		
Marital status	Married	206	55.2	47.50	12.17	-1.395**	0.164
	Single	167	44.8	49.28	12.36		
Number of children	None	195	52.3	48.84	12.64	1.178***	0.318
	1	82	22.0	49.13	11.64		
	2	83	22.3	46.07	12.21		
	3 and above	13	3.5	49.00	10.32		
Education	High school	8	2.1	52.25	11.46	0.444***	0.722
	Associate degree	43	11.5	47.02	11.30		
	Licence	183	49.1	48.25	12.43		
	Postgraduate	139	37.3	48.51	12.45		
Profession	Doctor	55	14.7	49.38	11.63	0.228***	0.923
	Nurse – Midwife	113	30.3	48.38	12.52		
	Physiotherapist	73	19.6	48.60	12.57		
	Dentist	88	23.6	47.56	13.33		
	Health technician	44	11.8	47.66	9.85		
Working in a pandemic hospital	Yes	176	47.2	48.98	12.36	1.019**	0.309
	No	197	52.8	47.68	12.19		
Chronic disease	None	308	82.6	48.05	12.30	1.885***	0.096
	Diabetes	7	1.9	48.71	4.39		
	Hypertension	15	4.0	43.60	13.54		
	Heart disease	10	2.7	56.60	12.42		
	Pulmonary disease	8	2.1	55.00	17.04		
	Other	25	6.7	48.56	9.34		
Use of medication	Yes	60	16.1	49.33	12.36	0.717**	0.474
	No	313	83.9	48.09	12.26		

*p<0.05, **Independent t-test, ***one-way ANOVA. Bold indicates statistically significant values.

the pandemic, and those who were worried about becoming infected and having infected people around them were also found to have higher levels of anxiety. In addition, the health care workers whose workload increased during the pandemic, those who were dissatisfied with their profession, those who wished to change their profession, and those who thought the COVID-19 precautions in their workplace were insufficient were also determined to have higher levels of anxiety.

In parallel with the results of our study, the vast majority of studies conducted during the COVID-19 pandemic have reported that female health care workers had higher levels of anxiety than their male counterparts[16,17]. This result was thought to be due to females taking on primary roles in the home (wife, parent) and internalizing the fear of contagion more[18,19]. In addition, when it is considered that more than 64% of this study sample was female, this result should be considered in this context. In a study by Santamaria et al, it was noted that together with the female gender, those of older age also had higher levels of anxiety[20]. In contrast, the results of this study found older age to be in negative proportion

Table 2. Comparison of the participants' Penn State Anxiety Total Scores according to the answers given to the questions about pandemic conditions.

Variables		n	%	X	SD	Test Value	p	Post-hoc
Need to be isolated from the family during the epidemic	Yes	322	86.3	48.81	12.24	2.071	**0.03***	
	No	51	13.7	45.00	12.01			
Working night shift at hospital	Yes	187	50.1	48.65	12.71	0.568	0.57	
	No	186	49.9	47.93	11.82			
Taking part in risky units when needed	Yes	200	53.6	49.29	12.14	1.701	0.09	
	No	173	46.4	47.13	12.35			
Any infected person in the environment	Yes	132	35.4	50.00	11.98	1.997**	**0.04***	
	No	241	64.6	47.35	12.35			
Lost a loved one during the COVID-19	Yes	24	6.4	46.33	17.09	-0.808	0.42	
	No	349	93.6	48.42	11.88			
Been infected during the pandemic	Yes	15	4.0	42.46	13.17	-1.883	0.06	
	No	358	96.0	48.53	12.19			
Job satisfaction	Very satisfied[1]	132	35.4	45.29	12.65	7.095***	**<0.001***	1<3, 2<3
	Somewhat satisfied[2]	159	42.6	48.54	11.38			
	Not satisfied[3]	57	15.3	53.92	13.32			
	Not sure[4]	25	6.7	49.68	8.10			
Worry about being infected	Yes	290	77.7	49.49	11.65	4.794	**<0.001***	
	No	56	15.0	41.48	13.48			
Thinking that Covid-19 precautions are sufficient	Yes[1]	109	29.2	44.62	11.72	7.608***	**0.001***	1<2
	No[2]	165	44.2	49.24	12.28			
	Not sure[3]	99	26.5	50.74	12.04			
Increase in the workload after the pandemic	Yes	224	60.1	49.67	12.26	2.698**	**0.007***	
	No	149	39.9	46.20	12.01			
Request to change profession	Yes	180	48.3	50.77	12.23	10.24***	**<0.001***	
	No	143	38.3	44.77	11.70			
	Not sure	50	13.4	49.42	11.82			
Difficulties in accessing personal protective equipment	Yes	182	48.8	49.51	12.46	1.887**	0.06	
	No	191	51.2	47.12	11.99			

*p<0.05, **Independent t-test ***One-way ANOVA. Bold indicates statistically significant values. [1,2,3,4] Multiple comparisons: Bonferroni (see *Post-hoc*).

Table 3. Results of multiple regression analysis: the effect of participants' answers to questions on Penn State Anxiety Total Scores.

Dependent variable	Independent variable	β	SE	t	p	F	Model (p)	R^2	Durbin-Watson
PSWQ score	Constant	38.176	1.976	19.315	**<0.001**	**6.769**	**0.000***	0.130	2.363
	Gender=Female	0.132	1.291	2.623	**0.009**				
	The need to be isolated from the family during the epidemic=Yes	0.014	1.851	0.262	0.794				
	Any infected person in the immediate environment=Yes	0.104	1.313	2.030	**0.043**				
	Job satisfaction = Not satisfied	0.123	1.839	2.281	**0.023**				
	Worry about being infected=Yes	0.202	1.502	3.955	**<0.001**				
	Thinking that COVID-19 precautions are sufficient=No	-0.050	1.295	-0.948	0.344				
	Increase in the workload after the pandemic=Yes	0.041	1.245	0.820	0.413				
	Request to change profession=Yes	0.109	1.348	1.990	**0.047**				

PSWQ: The Penn State Worry Questionnaire, *p<0.05. Bold indicates statistically significant values.

to anxiety. The reason for this difference could be, as stated by Laranjeira et al, that younger health care workers are assigned to active work in COVID-19 wards that require a heavy workload[21].

In parallel with our results, several previous studies have reported that health care workers experience fear of becoming infected because of their work and are worried about the risk of infecting family and friends. In a study conducted in Africa, Chersich et al. reported that health care workers caring for COVID-19 patients were faced with the anxiety of separation from their families and the loss of patients or colleagues. Limited intensive care units and lack of personal protective equipment, in particular, have also been reported to create anxiety between health care workers and their families[22]. The rapid spread of COVID-19 and the high morbidity and mortality rates could be another factor further increasing existing anxiety.

In a study by Mehta et al., the increased workload and lack of personal protective equipment were reported to increase the anxiety of health care workers and cause them to isolate from their families[23]. In another study, lack of personal protective equipment and other medical supplies was reported to create a primary source of anxiety for health care workers, and this could have destructive effects on the health care system[24]. In another study, it was reported that the fear of becoming infected increased anxiety in nurses and that this was associated with the wish to leave work[25]. Similar to these findings, the predominant factors increasing anxiety in health care workers in this study were seen to be an increased workload during the pandemic and insufficient precautions against COVID-19 in the workplace. Furthermore, the health care workers in this study with high levels of anxiety were found to be dissatisfied with the profession. Unlike the findings of previous studies, access to personal protective equipment did not increase anxiety. This difference could be attributed to this study data having been collected in the period of August–October when health care workers did not experience problems in accessing protective equipment, unlike the early stages of the pandemic.

Strengths and limitations

One of the significant strengths of this study is that the anxiety states of different health care professionals were evaluated. Another strength was that health care professionals working in different health care centers across the country were included. Moreover, the effect on anxiety of several parameters related to working conditions during the COVID-19 pandemic was evaluated. Although it seems that beneficial results have been obtained with vaccinations and the workload of health care professionals has been reduced, the COVID-19 pandemic is continuing with the effect of different variants of the virus. Taking this into consideration, the most significant limitation of this study was the limited data collection dates and that a long-term follow-up was not included.

CONCLUSIONS

The anxiety levels of health care workers, who play a key role in the struggle against the COVID-19 pandemic, can be increased by young age, low experience, female gender, fear of being infected and contagion, increased workload, insufficient precautions against COVID-19 in the workplace, and dissatisfaction with the profession. For a more effective fight against COVID-19, it is necessary to identify situations that cause anxiety in health care workers. It is very important to develop coping strategies to eliminate these situations and to provide psychological support for health care workers during the pandemic. However, further studies with larger samples are recommended to examine the long-term effects of crises and epidemics on health care workers.

ETHICS COMMITTEE APPROVAL

Permission to conduct the study was obtained from the Istanbul University-Cerrahpasa Medical Research Ethics Committee and the Ministry of Health (Ethics Committee Decision No: 60116787-020/41133). Consent to participate was obtained from the study subjects by adding a Voluntary Participation Form to the online questionnaire prepared through the interface of Google Forms, and the consent was recorded on the Internet.

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AUTHORS' CONTRIBUTIONS

AC, EZ, SOA, BBC: Conceptualization, Methodology, Data curation, Formal Analysis, Investigation, Writing – original draft, and Writing – review & editing. **BBC:** Conceptualization, Methodology, Project administration, Supervision, and Validation.

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ORIGINAL ARTICLE

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Effects of imipenem combined with glutamine in the treatment of severe acute pancreatitis with abdominal infection in mainland China: a meta-analysis

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SUMMARY

OBJECTIVE: The aim of this study was to explore the efficacy of imipenem combined with glutamine in the treatment of severe acute pancreatitis with abdominal infection in mainland China using meta-analysis.

METHODS: We searched China National Knowledge Network, Wanfang Medical Network, Chinese Science Citation Database, PubMed, and Embase Databases for publications of imipenem combined with glutamine in the treatment of severe acute pancreatitis abdominal infection. The search time limit was from the establishment of the database to April 10, 2021. Stata software version 12.0 was used for statistical analysis; the combined effect size odds ratio and standardized mean difference values were calculated for the count data and measurement data, respectively; and the heterogeneity test was performed in this study.

RESULTS: A total of five randomized controlled trials were included. A total of 499 cases were included, with 251 in the observation group and 248 in the control group. Meta-analysis results showed that the efficacy of imipenem combined with glutamine in the treatment of severe acute pancreatitis with abdominal infection was significantly better than that of imipenem alone (odds ratio=0.78, 95%CI 0.71–0.86, p=0.040).

CONCLUSION: Imipenem combined with glutamine can significantly improve the efficacy in the treatment of severe acute pancreatitis with abdominal cavity infection.

KEYWORDS: Pancreatitis. Imipenem. Glutamine. Intraabdominal infection. Meta-analysis.

INTRODUCTION

Pancreatitis is a disease of the pancreas caused by the self-digesting effect of trypsin. The patient with pancreatitis has edema, congestion, or bleeding or necrosis. Acute pancreatitis is an inflammatory reaction that causes pancreatic enzymes to digest, edema, hemorrhage, and even necrosis after the activation of pancreatic enzymes in the pancreas. Clinical patients are characterized by acute upper abdominal pain, nausea, vomiting, fever, and increased blood pancreatin. The severity of the disease varies. Severe acute pancreatitis (SAP) is a special type of acute pancreatitis. SAP is an acute and severe disease in which the activation of pancreatic enzymes in the human body causes local inflammation of the pancreas and damages the functions of many organs. This disease is accompanied by systemic and local complications, accounting for 10–20% of the entire acute pancreatitis. In the 1980s, most cases died in the early stages of the disease. Until the past 10 years, with the advancement of SAP treatment technology, the cure rate has increased, but the overall mortality rate is still as high as 17%. According to relevant data, more than 80% of SAP patients are clinically affected by overeating, long-term alcohol abuse, and biliary diseases. This disease has the characteristics of rapid onset, rapid development, dangerous condition, complications, and high mortality rate, which seriously threatens the lives and health of people[1].

Effective clinical treatment to delay the progression of the patient's condition has become a key research topic for medical workers. The number of SPA patients who died of acute respiratory distress syndrome and shock decreased year by year, while the number of patients who died of an abdominal infection that could not be controlled earlier increased year by year. The patient's abdominal infection is closely related to the necrosis of the pancreas and surrounding tissues. The larger the range of necrosis, the higher the probability of infection. Therefore, it is necessary to take effective treatment methods in time to control the abdominal cavity infection of patients in order to improve the clinical efficacy of the treatment of SAP[2].

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A single clinical trial is difficult to confirm certain effects that may be important to clinicians because of the small sample. Meta-analysis is a systematic evaluation method that synthesizes various published research data and analyzes the combined effect of the extracted data. This method can increase statistical power and conforms to objective laws. It is a huge advancement, which is completely consistent with the idea of evidence-based medicine[3].

This study aims to systematically evaluate the therapeutic effect of imipenem combined with glutamine on SAP with abdominal cavity infection through the method of evidence-based medicine, so as to provide a reference for future clinical medication plans.

METHODS

Search strategy for publications

We used the terms "imipenem," "glutamine," "pancreatitis," "acute pancreatitis," "severe acute pancreatitis," "abdominal infection," "China," and "mainland" to search publications in the Chinese databases of China National Knowledge Network (CNKI), Wanfang Medical Network, and China Science Citation and the English databases of PubMed and Embase. The search date was from the establishment of the database to April 10, 2021.

Participants

The inclusion criteria were as follows: ① those who met the diagnostic criteria for SAP[4]; ② those who had clinical symptoms of abdominal infection, such as abdominal pain, abdominal muscle tension, abdominal wall tenderness and rebound pain, and abdominal effusion; ③ those who underwent diagnosis by serum enzymology, imaging, and abdominal puncture; and ④ those who had not taken glutamine or imipenem 1 month before treatment. The research design is a clinical randomized controlled trial (RCT). The observation group comprises patients who received combined imipenem and glutamine, and the control group comprises those who received imipenem only. The exclusion criteria were as follows: ① non-RCTs, ② unclear number of effective cases, ③ unclear efficacy indicators or criteria; and ④ animal experiments, reviews, or case reports.

Efficacy evaluation

The efficacy of the cases was classified in three ways: ① significantly effective: Clinical symptoms and signs of the patients have basically disappeared; the abdominal infection symptoms have been significantly relieved; the serum inflammatory factor levels and blood biochemical index levels have returned to normal; and imaging examinations show that the pancreas has returned to normal; ② effective: Clinical symptoms and signs of the patients have been reduced, the symptoms of abdominal infection have been improved, the levels of serum inflammatory factors and blood biochemical indicators have been significantly improved, and the results of imaging examinations have shown that the pancreatic images have been significantly improved; and ③ invalid: There is no change in clinical symptoms and abdominal infection, and there is even a tendency to worsen. The total number of effective cases is the sum of significantly effective and effective cases.

Data extraction and literature quality evaluation

The screening of literature and data extraction were carried out independently by two researchers. The main content of data extraction included ① basic information of the literature; ② evaluation index of literature quality; and ③ clinical efficacy result index.

The third researcher resolved the differences that had arisen.

The quality of the literature was assessed using the Jadad quality scoring criteria, including whether randomization (2 points), blinding (2 points), and withdrawal (1 point) were described.

Statistical analysis

Stata software version 12.0 was used in this study. The forest diagram and the funnel diagram were drawn. When the curative effect index was countable data, the odds ratio (OR) value was used as the comprehensive effect. For continuous data, the standardized mean difference (SMD) value was used as the comprehensive effect, and the 95% confidence interval (95%CI) was used for interval estimation. The Q-test was used to analyze the heterogeneity of the included studies. I^2>50% and p<0.05 were considered heterogeneous, and the random-effects model was used to merge the effect size in the meta-analysis. Otherwise, the fixed-effects model was used. The funnel plot and Egger's test were used to analyze the publication offset. A p<0.05 was considered statistically significant.

RESULTS

Basic information of the included publications

A total of 5 RCTs and 499 patients, with 251 in the observation group and 248 in the control group, were finally included. In the process of document screening, 25 publications were initially retrieved. Then, we excluded 16 reviews and non-RCTs after reading the title and abstract, and further excluded 4 articles (those with incomplete data and co-administration of other drugs) after reading the full text. Finally, five publications were

included in this study. The basic information of the included literature is provided in Table 1.

Meta-analysis results of efficacy

The result of the heterogeneity test showed that there was no heterogeneity (I^2=16.5%, p=0.309) and, therefore, the fixed-effects model was selected. Meta-analysis results showed that the efficacy of imipenem combined with glutamine was significantly better than that of imipenem alone (OR=0.21, 95%CI 0.15–0.30, p<0.001) in the treatment of SAP with abdominal infection. The forest plot is shown in Figure 1.

Table 1. Basic information of the included publications.

First author	Year	Country	Area	Interventions		Number of cases (observation group/control group)	Duration (day)	Jadad score
				Observation group	Control group			
Wang XT	2019	China	Beijing/ Haerbin	Imipenem (1 g bid)+glutamine (1.5–2.0 mL/kg qd)	Imipenem (1 g bid)	53/50	14	3
Jin ZQ	2018	China	Hubei	Imipenem (1 g bid)+glutamine (2.0 mL/kg qd)	Imipenem (1 g bid)	46/46	14	4
Jiang DQ	2017	China	Hebei	Imipenem (1 g bid)+glutamine (1.5–2.0 mL/kg qd)	Imipenem (1 g bid)	51/51	14	3
Hong L	2017	China	Jiangsu	Imipenem (1 g bid)+glutamine (20 g qd)	Imipenem (1 g bid)	58/58	14	4
Xu B	2019	China	Heilongjiang	Imipenem (1 g bid)+glutamine (2.0 mL/kg qd)	Imipenem (1 g bid)	43/43	14	3

bid: two times daily; qd: once a day.

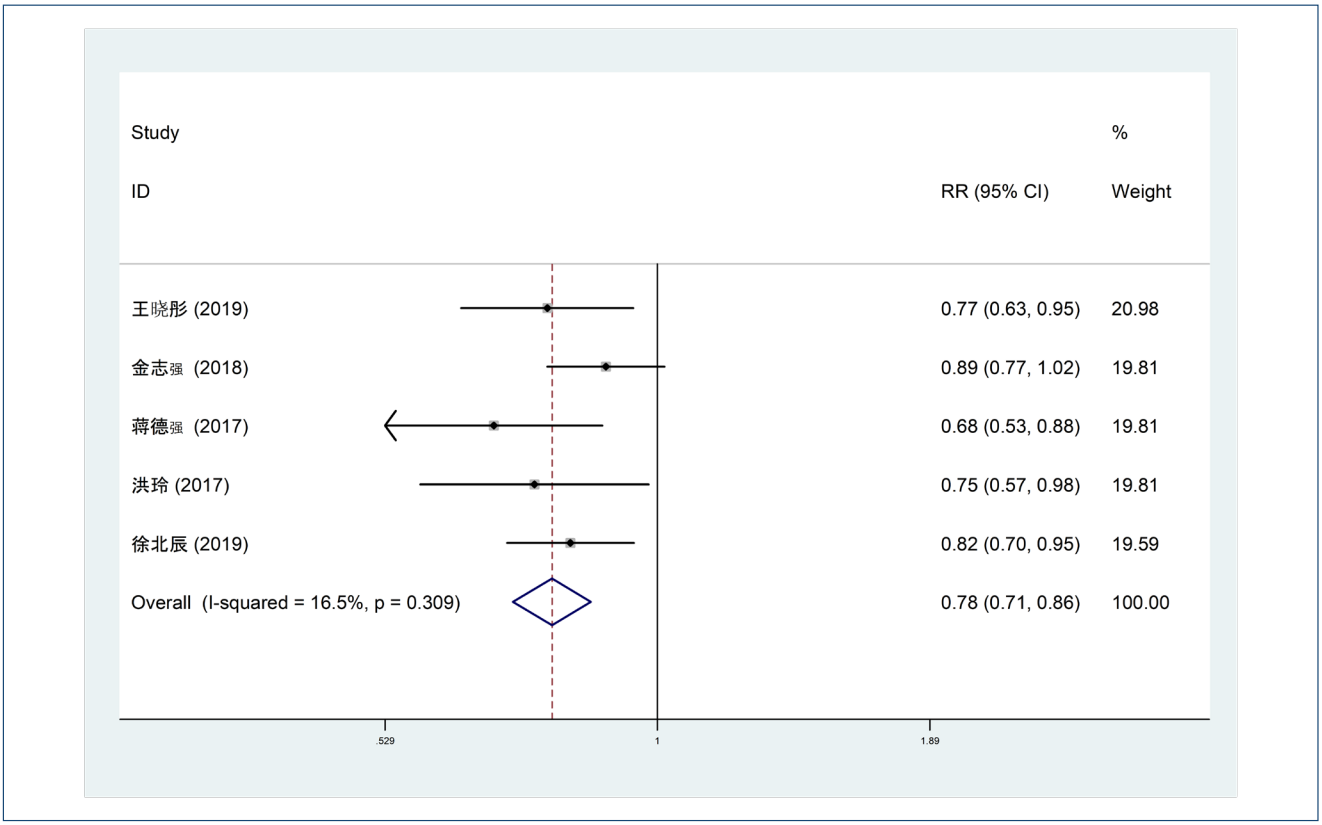

Figure 1. Forest plot of the efficacy of imipenem combined with glutamine.

Adverse reactions

Since only one of the included literature involved adverse reactions, the effect size was not combined. These adverse reactions include acute respiratory distress syndrome (0 vs. 1), renal insufficiency (1 vs. 2), shock (1 vs. 1), coagulation dysfunction (2 vs. 1), and intestinal paralysis (2 vs. 0) between the two treatment groups (observation group vs. control group).

Publication bias

Egger's test results showed that the hypothesis test results of publication bias were statistically significant (t=3.48, p=0.040). Publication bias can be considered to exist. The funnel plot is shown in Figure 2.

DISCUSSION

This study is the first to conduct a meta-analysis of imipenem combined with glutamine in the treatment of SAP with abdominal infection from the perspective of evidence-based medicine in mainland China. The included literature covers Beijing, Harbin, Hubei, Hebei, Jiangsu, Heilongjiang, and other regions of China. The results showed that imipenem combined with glutamine is significantly better than imipenem alone in the treatment of SAP with abdominal infection, which will provide great help to clinicians in medication.

SAP is a special type of acute abdomen characterized by persistent organ failure, which lasted for more than 48 h. A large-scale, multi-center, retrospective study showed that the fatality rate of SAP within 1 week of the course of disease was even as high as 67%[5,6]. SAP is usually treated by routine internal medicine combined with β-lactam antibiotics. This treatment method has a significant positive effect on alleviating the clinical symptoms of patients and achieving a cure effect. However, the long-term practice has confirmed that this treatment method only focuses on symptomatic treatment and lacks targeted treatment for the causes of abdominal infection. Therefore, the biochemical indicators of SAP patients with abdominal infection and the recovery of abdominal infection symptoms are slow, and the clinical treatment effect is not good[7,8].

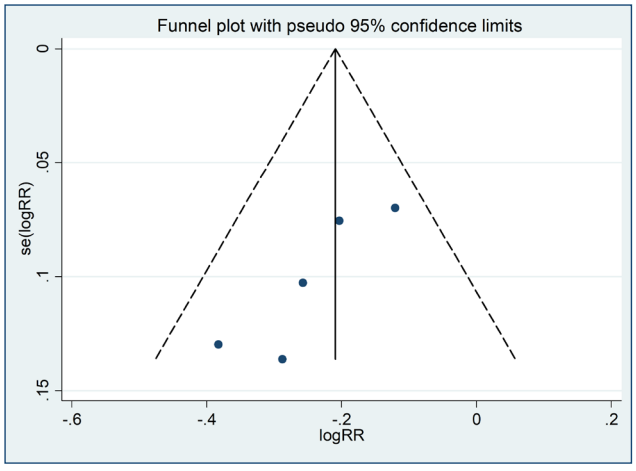

Figure 2. Funnel plot of the efficacy of imipenem combined with glutamine.

Abdominal infection and tissue damage in SAP patients are closely related to the abnormal expression of serum inflammatory factors. Tumor necrosis factor α (TNF-α) is not only the first typical cytokine to cause an increase in the onset of SAP but can also effectively induce the production of interleukin-6 (IL-6), IL-8, and other inflammatory factors, causing the release of inflammatory mediators, thereby aggravating the symptoms of abdominal infection in SAP patients[9]. Moreover, abdominal cavity infections are mostly caused by the lack of amino acids in the human body due to fasting during the treatment of SAP patients. Due to increased intestinal wall permeability, intestinal mucosal atrophy, and villi loss, the resident flora in the intestine invades the abdominal cavity[10].

The early-stage infection in SAP patients is usually caused by single bacteria. With the gradual development of the disease, infections caused by the joint action of multiple bacteria are more likely to appear in the later stage. Gram-negative bacteria are the main infection bacteria of patients, such as *Enterococcus*, *Escherichia coli*, and *Klebsiella*[11]. The severity of the disease is directly related to the complexity of the infection. It may be a bacterial or fungal infection, and there is also the possibility of double infection[12]. Imipenem is a kind of β-lactam antibiotics, with very strong effect, especially for Gram-positive bacteria and anaerobic bacteria. Therefore, imipenem is mainly appropriate for treating abdominal infection symptoms caused by a variety of pathogens[13].

Glutamine is a non-essential amino acid secreted by skeletal muscle. It is one of the components of parenteral nutrition support, which can effectively repair the digestive tract. The mechanism is to inhibit the atrophy of the intestinal mucosa, reduce the permeability of the intestinal mucosa and the release of endotoxin, improve the intestinal microenvironment, and maintain the immune function of the intestine[14].

After the diagnosis of co-infection in patients, even if the pathogen culture and drug sensitivity test results have not been obtained, antibacterial drugs should be given in time, and the data obtained in this study have certain reference value for empirical medication. In fact, the key to improving the

treatment effect of patients with SAP lies in the early diagnosis of the disease and the timely adoption of appropriate treatments to prevent abdominal infections[15,16]. After all, prevention is better than cure.

There were several limitations in this study, including a small number of included literature and cases and inconsistent medication time. In addition, there are differences in the physiological mechanisms of Asian and European populations, while the cases included in this article were only from China. RCTs with larger sample sizes are suggested in the future studies. However, our results have enriched the SAP treatment research system.

CONCLUSION

Imipenem combined with glutamine can significantly improve the efficacy in the treatment of SAP with abdominal cavity infection.

AUTHORS' CONTRIBUTIONS

SZ, LRJ, CH: All authors contributed equally to this work. Their contributions include Conceptualization, Data curation, Formal Analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, and Writing – review & editing.

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ORIGINAL ARTICLE

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Wnt3a but not CDX-2 expression is associated with differentiated thyroid cancer

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SUMMARY

OBJECTIVE: Thyroid neoplasm incidence has increased worldwide, mostly due to the advancements in medical imaging and screening rates. The aberrant Wnt/β-catenin pathway has been identified as a key mechanism, and it has also been related to the metastatic activity of differentiated thyroid cancer. We aimed to verify the difference in the expression of Wnt3a, a canonical activator of the β-catenin signaling, and CDX-2, a transcription factor upregulated by Wnt/β-catenin pathway, in multinodular goiter and differentiated thyroid cancer and to determine their prognostic value.

METHODS: We included 194 thyroid tissue surgical specimen and their clinicopathological data: study group (differentiated thyroid cancer, n=154) and control group (multinodular goiter, n=40). Immunohistochemistry (IHC) was performed on formalin-fixed, paraffin-embedded tissue by the primary antibodies Wnt3a and CDX-2.

RESULTS: High Wnt3a expression was significantly associated with differentiated thyroid cancer (p=0.031). CDX-2 was negative in all differentiated thyroid cancer cases (100%) and also in multinodular goiter. Wnt3a expression was significantly associated with tumors ≤20 mm (p=0.044) and with the absence of capsule invasion (p=0.031). The multivariate analyses suggested that older age (≥55), independent of capsular invasion and tumor size, was an independent prognostic factor for Wnt3a expression (p=0.058).

CONCLUSIONS: Wnt3a expression but not CDX-2 is correlated with differentiated thyroid cancer samples in comparison to multinodular goiter. Although its prognostic value was limited to tumor size and capsule invasion, a combined model in a panel of immune markers can add accuracy in the classification of challenging thyroid follicular-derived lesions.

KEYWORDS: Wnt3A protein. CDX2 protein. Thyroid cancer. Goiter. Biomarker.

INTRODUCTION

Differentiated thyroid carcinoma (DTC), the most common endocrine malignancy, detected mainly after the formation of a cervical nodule, has a low mortality rate. However, some rare histopathological variants of DTC are associated with the more aggressive disease with increased risk of tumor-related death[1]. Less is known about reliable factors that could not only predict outcomes in DTC but also help to differentiate benign proliferated cells from malignant ones[2]. Due to the increasing use of powerful imaging diagnostic tools, a great number of low-risk diseases or benign thyroid nodules are being treated with unnecessary aggressiveness, also yielding thyroid cancer over-diagnosis and ramping up comorbidities associated with its overtreatment[3].

The thyroid gland is a relatively dormant organ, and the control of regeneration of a thyroid gland is complex. It is uncommon for a benign thyroid adenoma to evolve toward a carcinoma. It seems that most carcinomas are malignant from the onset. Initiation and progression of thyroid cancer involve multiple genetic disruptions, of which mutations leading to the activation of the MAPK, PI3K/AKT, and Wnt/β-catenin signaling pathways are the most explored[4]. Aberrant positive staining for β-catenin in DTC and multinodular goiter (MNG) has been documented[5]. Wnt proteins, closely controlling Wnt/β-catenin pathways activation, contribute to the homeostasis of several tissues of epithelial origin and are implicated in vital cellular functions like stem cell regeneration, cell survival, and organogenesis[6]. Wnt1, Wnt3a, Wnt7a, or Wnt10b induces the accumulation of intracellular β-catenin by stimulating the inactivation of the formation of the β-catenin destruction complex, therefore leading to a marked cell proliferation[7].

CDX-2, a transcription factor of the caudal homeobox family, upregulated by Wnt3a, plays an important role in the differentiation and polarization of epithelial cells. Given that the thyroid gland develops from foregut endoderm, the inhibition of endoderm posterior pattern that leads to promoting

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anterior foregut polarization is necessary to driving thyroid progenitor cells to differentiated follicular epithelial cells[8]. This is accomplished, in part, by CDX-2 expression[9].

The ability of the adult thyroid gland to regenerate in response to injury represents an important homeostatic process. The representative Wnt3a and CDX-2 interplay is illustrated in Figure 1.

In DTC, CDX-2 nuclear expression has been described in cases of columnar cell variant, with still no definition about its relevance in thyroid tumor aggressiveness[10,11]. To date, it has not been studied in MNG, hence, its absence correlates with less histological differentiation and advanced staging in colorectal malignant tumors shedding light on a tumor suppression function[12].

The expression of CDX-2 in association with Wnt3a in both MNG and DTC has not been studied previously.

This research aimed to compare the immunostaining of MNG and well-differentiated follicular thyroid neoplasms for Wnt3a and CDX-2 and to determine their prognostic value in DTC.

METHODS

This is a cross-sectional retrospective study, carried out at University Evangelical Mackenzie Hospital, in Curitiba, PR, Brazil. Total thyroidectomy (TT) was performed between the years 2002 and 2017. DTC's demographic information, clinical data, treatment, and follow-up were collected. Two pathologists reviewed the blocks.

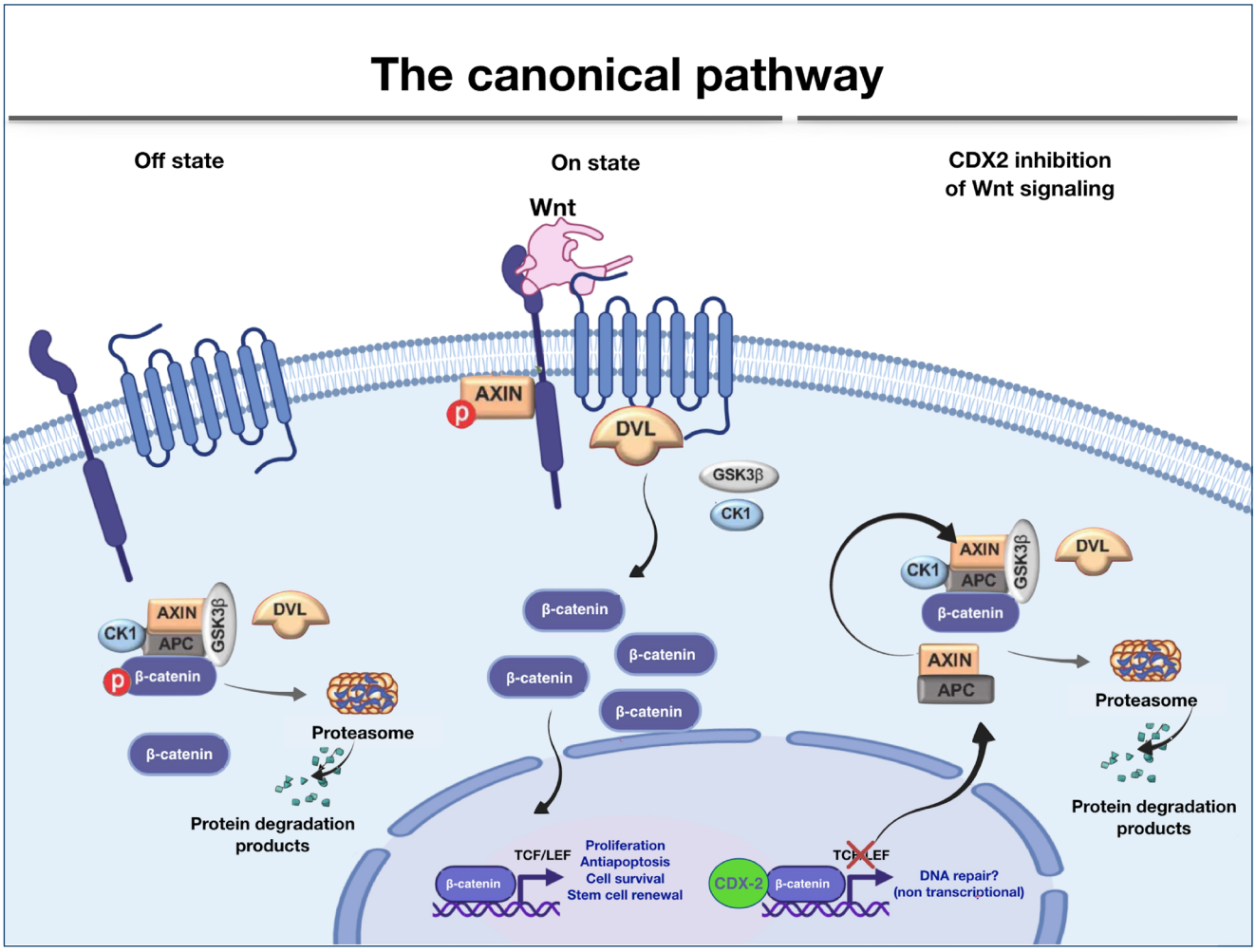

Figure 1. Schematic representation of the interaction between Wnt3a and CDX-2 during the genesis of the thyroid and cellular regeneration. (1) Off state: In a dormant state, when the cells are not dividing, the β-catenin is degraded. (2) On state: Wnt3a stimulates the inactivation of the formation of the β-catenin destruction complex (formed by casein kinase 1 – CK1, glycogen synthase kinase 3 – GSK3, axin protein, and APC protein), which causes intracellular accumulation of β-catenin. This accumulation leads to the activation of target genes of the Wnt/β-catenin pathway, responsible for controlling cell survival, proliferation, and stem cell renewal. (3) CDX inhibition of Wnt signaling: the CDX2 transcription factor, important for the morphogenesis of the thyroid follicular epithelial cells, is believed to act as a blocking factor of the Wnt/β-catenin signaling pathway synergistically. Created with BioRender.com.

The sample consisted of 154 patients with the diagnosis of DTC and 40 patients with MNG, selected from the Anatomic Pathologic database. The demographic information, clinical data, treatment, and follow-up of patients with DTC were collected between January 2018 and December 2019. TT was performed between the years 2002 and 2017.

Formalin-fixed, paraffin-embedded thyroid surgical specimens were sent for immunostaining. For immunohistochemical (IHC) evaluation, multi-sample blocks were built using the Tissue Tek Quick-Array™ handpiece. Coupled clamps allowed to extract sections of 1–3 mm each from the most representative area of the tumor. Each multi-sample block yielded 5 µm thickness slides that were subsequentially submitted to the immunoperoxidase technique performed using the Benchmark Ultra™ instrument. Incubation with the primary antibodies lasted 20 min at room temperature. The primary antibodies used were membrane Wnt3a (GeneTex, CA, USA; diluted 1:100) and nuclear CDX-2 (CellMarque, CA, USA; diluted 1:200). Amplification was conducted by Ultraview Universal DAB Detection Kit® following the manufacturer's instructions. The immunostained TMA sections were evaluated and scored under a light microscope by two pathologists at different time points. Immunostaining for nuclear CDX-2 was scored in a three graded scale: score 0, weak staining in <10% of the tumor cells; score 1, moderate staining ≥10–75% of the tumor cells, and score 2, strong staining in ≥75% of the tumor cells. Positive membrane Wnt3a was considered when marked in ≥10% of the tumor cells.

Informed consents were collected under Institutional Review Board-approved protocols of the Hospital Universitário Mackenzie do Paraná (nº 1.999.671) in Curitiba, Paraná, Brazil.

Statistical analysis

Continuous and categorical variables were compared using the Student's t-test and chi-square test. For the multivariate analysis, the logistic regression model was adjusted including the ones with value <0.25. Wald test was used to determine the significance of the variables, and the estimated association measure used was the odds ratio. The $p<0.05$ indicated a statistical significance. Data were analyzed using Stat/SE version 14.1 (StataCorpLP, USA).

RESULTS

Of the 154 patients with DTC, 125 (81.2) were woman, with a mean age of 45.7±13.53 years. Papillary thyroid cancer (PTC) was the most common histological variant (n=112, 72.7%). These data are presented in detail in Table 1. Most cases were classified as TNM class I (n=132, 85.7%). Low-risk MRSS ATA 2009 (Modified Risk Stratification System from American Thyroid Association Guidelines 2009) was diagnosed in the majority (n=88, 57%). Central lymphadenectomy was performed in 104 (67.5%) cases, and bilateral cervical exploration was performed in 21 (13%) cases. A total of 22% (n=35) of patients did not receive radioiodine remnant ablation (RRA), and 7.1% (n=11) and 68% (n=105) did undergo either low-dose (30 mCi) or high-dose RRA (100–200 mCi), respectively. Group MNG, mean age 59.5 years, was mostly composed of women (92.5%). Although gender distribution was not different, age was statistically distinct between groups: 59.5 years in MNG and 45.2 years in DCT ($p<0.01$).

Table 1. Baseline characteristics of 154 patients with MRSS ATA 2009: modified stratification system.

Age (years)	
Mean±SEM	45.7±13.53
Median (range)	43.4 (19.2–85.2)
Histology, n (%)	
PTC classic variant	112 (72.7)
PTC follicular variant	32 (20.8)
PTC oncocytic variant	3 (1.9)
PTC tall cell variant	2 (1.3)
Follicular	5 (3.2)
Cervical lymphadenectomy, n (%)	
No	29 (18.8)
Central	104 (67.5)
Central and lateral	21 (13.0)
Bilaterality, multifocality, and capsular invasion, n (%)	
Bilateral	38 (24.7)
Multifocal	57 (37)
Capsular invasion	47 (30.5)
TNM stage, n (%)	
I	132 (85.7)
II	11 (7.1)
III	10 (6.5)
IVc	1 (0.6)
MRSS ATA 2009, n (%)	
Low	88 (57.1)
Intermediate	45 (29.2)
High	21 (13.6)
^{131}I ablative dose (mCi)	
Mean±SEM	86.7±57.8
Median (range)	100 (0–200)

CDX-2 expression was negative in 145 (94%) patients, and the results were inconclusive in 9 (5.8%) patients. Group MNG also showed the absence of CDX-2 expression in all cases.

The expression of Wnt3a in the group MNG was positive in only 7 (17.5%) cases and negative in 33 (82.5%) cases. The demographics and clinical features of the patients in the DTC study group and MNG are presented in Table 2.

Wnt3a expression was inconclusive in 33 (21.4%) cases. Among the other 121 cancerous tissues, 45 (37.2%) cases showed high Wnt3a expression. A significant difference was detected between DTC and MNG (p=0.031). Wnt3a expression was significantly associated with smaller tumors (p=0.044) and the absence of capsule invasion (p=0.031).

In a multivariate analysis, tumor size was significantly associated (p=0.024) with Wnt3a expression, while capsule invasion presented only a trend association (p=0.052). Age ≥55 years old, independent of capsular invasion and tumor size <2 cm, also showed a statistically significant trend (p=0.058).

DISCUSSION

In this research, we found increased expression of Wnt3a in DTC compared to the significant lower expression in MNG, indicating that IHC analysis of Wnt3a staining is a valuable tool for differentiating DTC from proliferative benign thyroid tumors. To the best of our knowledge, this is the first time that these markers were studied comparing thyroid carcinomas and benign thyroid hyperplasia blocks. Similarly, a study investigated Wnt3a expression in PTC samples and compared it to their paracancerous tissues[13]. In this case, the results of IHC showed that the positive expression rates of Wnt3a in PTC tissues were significantly higher. Interestingly, in their report, the expression of Wnt3a is correlated with TNM stage, differentiation, extramembranous invasion, and lymph node metastasis, but not with tumor size. In our study, however, Wnt3a overexpression was correlated with less aggressive characteristics, according to TNM-AJCC, in a significant manner. It was detected more in small tumors, less than 2 cm, and in those without capsule invasion.

Table 2. Characteristics of subjects and Wnt3a expression in multinodular goiter and differentiated thyroid carcinoma.

Variable		Groups		p-value
		MNG (n=40)	DTC (n=121)	
Age	Years	59.5±14.1	45.2±13.1	<0.001
Gender	Female	37 (92.5)	100 (82.6)	0.199
Wnt3a	Negative	33 (82.5)	76 (62.8)	0.031
	Positive	7 (17.5)	45 (37.2)	

These results highlight a possible role of the canonical activator Wnt3a acting as a tumor suppressor as reported in some other tissues. In non-solid neoplasms, Nygren et al. showed B-cell precursor acute lymphoblastic leukemia (B-ALL) cell death induction by Wnt3a[14]. Also, by activating canonical Wnt signaling in ALL cells, the Wnt3a is known to inhibit the proliferation of B-ALL cell lines[14]. In melanoma, Wnt3a has been related to enhanced apoptosis[5], and in hepatocellular carcinoma, it has been shown to improve hypoxia-induced epithelial–mesenchymal transition (EMT)[15].

Perhaps, the uncommon aggressiveness and low mortality rate seen in DTC have deployed researchers toward studying more aggressive tumors, in an attempt to unveil the Wnt/β-catenin pathway role in cancer. This could explain why the literature is scarce relating Wnt family members to DTC.

In one of these few reports, using cell culture, Cassinelli et al. showed that in human primary thyrocytes, RET/PTC1 promotes β-catenin transcriptional activity to drive thyrocyte neoplastic transformation, indicating that RET–PTC fusions also trigger the Wnt pathway in an independent manner of Wnt members activation[16]. Recently, in a study with co-cultured cells using thyroid cancer cells and alternatively activated cells-like tumor-associated macrophages, the knockdown of Wnt3a reduced the proliferation and migration of thyroid cancer cells[17]. Even though these findings might oppose to those of ours, it is important to note that this study was performed using cell culture, which is done in an artificial environment that does not always reproduce the in vivo scenario.

Taking all these data together, it could be possible that Wnt3a expression, correlated to malignant tissues with better outcomes, was lost as an epiphenomenon of tumor dedifferentiation and, therefore, was not detected in some cases, rather than being a true pathogenic factor.

In our groups, CDX-2 expression was negative in all benign and in DTC samples. To date, CDX-2 expression has been seen only in tumors with columnar cell[18] and cribriform morular variant histology, the former associated with more aggressive behavior[19]. Since our cohort was composed only of one representative case of each above-mentioned variant and both were classified as low-risk cases, the absence of CDX-2 expression was predictable.

There are some limitations in our study. The non-neoplastic thyroid samples were not representative of other thyroid hyperplasia or even normal thyroid tissue, to better understand the above-mentioned markers. We also could not determine whether the Wnt3a expression is prognostic or a predictive factor due to the indolent behavior of most of our cases.

CONCLUSION

This study shows that Wnt3a expression but not CDX-2 is correlated with DTC samples in comparison to MNG. Although its prognostic value was limited to tumor size and capsule invasion, a combined model in a panel of immune markers can add accuracy in the classification of challenging thyroid follicular-derived lesions.

AUTHORS' CONTRIBUTIONS

GLKB: Conceptualization (Equal), Formal Analysis (Lead), Investigation (Lead), Methodology (Equal), Project administration (Lead), Resources (Lead), Supervision (Lead), Writing – original draft (Lead), Writing – review & editing (Lead). **CAPMR:** Funding acquisition (Lead), Project administration (Equal), Supervision (Equal). **HDH:** Data curation (Equal), Investigation (Equal). **VYH:** Data curation (Equal), Investigation (Equal). **MAKZ:** Data curation (Equal), Investigation (Equal). **IP:** Data curation (Equal), Investigation (Equal). **GB**: Project administration (Equal), Writing – original draft (Equal), Writing – review & editing (Equal). **LMC:** Conceptualization (Equal), Funding acquisition (Equal), Project administration (Equal), Resources (Supporting).

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ORIGINAL ARTICLE

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Does focal heterogeneity affect survival in postoperative ipsilateral multifocal and multicentric breast cancers?

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SUMMARY

OBJECTIVE: In multicentric/multifocal breast tumors, there may be immunological and histological differences between foci that may affect survival and treatment choice. We aimed to evaluate the effect of focal heterogeneity seen in multicentric/multifocal breast tumors on survival.

METHODS: We retrospectively collected and analyzed the clinicopathological data of 89 female patients with multifocal/multicentric breast cancer, whose surgical and medical treatment was completed and who were followed up for 5 years.

RESULTS: Of all patients, 29.2% (26/89) were heterogeneous. Heterogeneity of these foci was as follows: histologic heterogeneity of index foci (mix type): 15.7% (14/89), histologic heterogeneity of inter-foci: 7.9% (7/89), and immunohistochemical heterogeneity of inter-foci: 10.1% (9/89). When additional foci were evaluated, oncological therapy was changed for 3 (3.3%) of 89 patients. Heterogeneity does not have a significant (p>0.05) effect on recurrence and survival in multicentric/multifocal breast cancers. Pathological N stage is an independent risk factor for disease-free survival (hazard ratio=2.29, 95% confidence interval=1.39–3.76, p=0.001).

CONCLUSIONS: In multifocal/multicentric breast cancers, less than 4% of patients may experience heterogeneity requiring change in the therapeutic decision. However, heterogeneity does not have a significant effect on recurrence and survival in multifocal/multicentric breast cancers. The pathological N stage is an independent risk factor for disease-free survival.

KEYWORDS: Breas cancer. Multifocal. Multicentric. Tumor heterogeneity. Survival.

INTRODUCTION

Invasive ductal carcinoma (IDC) is the most frequently diagnosed and the most common type of breast cancer among women. In the latest classification of the World Health Organization, at least 17 different special histological types have been described, which constitute 25% of all breast cancers. Histological types are associated with the morphological and cytological features of the cell, derived from the growth pattern of breast cancer[1]. Depending on different histological methods employed and the cases selected, 6–75% of these tumors are multicentric (MC) or multifocal (MF) breast tumors[2].

The presence of multiple foci on the same quadrant or different quadrants of breast is defined as MC/MF[3]. The development of MF/MC breast cancers may be explained through two mechanisms in the literature. The first mechanism (polyclonal) may occur as multiple independent tumor foci. In these foci, different phenotypes may occur due to underlying molecular changes. The second mechanism (monoclonal) may be the genetic or phenotypic change in the foci during the intramammary spread of the tumor or the progression of the tumor[4]. In case of multiple breast cancers, there may be different histological types and/or histological grades between tumors in 3–37.5% of cases[5]. This situation may affect survival and treatment choice[6].

We aimed to investigate the effect of focal heterogeneity on 5-year disease-free survival (DFS) in MC/MF breast cancers because there is limited number of publications in the literature regarding intra- and inter-focal heterogeneity in MC/MF breast tumors.

METHODS

Ethical approval

Local ethics committee approval (dated November 13, 2020, Decision no.: 2588) was obtained.

Patient selection

Inclusion criteria: The patient files of Health Sciences University, Istanbul Training and Research Hospital, were retrospectively scanned and 89 female patients who underwent operation

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between January 1, 2010 and January 1, 2015, had surgical, immunohistochemical, and histopathological results [i.e., estrogen receptor (ER), progesterone receptor (PR), human epithelial growth factor receptor-2 (HER-2), proliferation index (KI-67)] for MC/MF breast cancer and who were given postoperative complementary medical treatments and followed up were identified and included in the study.

Exclusion criteria: Male patients; patients with preoperative stage 4 metastatic breast cancer, unifocal breast cancer, or bilateral breast cancer; and patients who received neoadjuvant chemotherapy, who did not undergo surgery and who did not have immunohistochemical and histopathological results, and follow-up records were excluded from the study.

Immunohistochemical subtypes classification

ER, PR (if ER or PR ≥1%, positive; if ER or PR <1%, negative), and HER-2 [HER-2 score=1–0, negative; HER-2 score=3, positive; and HER-2 score =2, positive which was tested using the Silver-enhanced in situ hybridization (SISH)] were accepted. The recommendations in the St. Gallen International Consensus Guidelines were taken into consideration in the immunohistochemical classification of subtypes.

Luminal A: ER+, PR+, HER-2-, Ki67<20% and/or PR≥20%, and/or low grade (G1/2).

Luminal B: ER+, PR+/-, HER-2-, Ki67≥20% and/or PR<20%, and/or high grade (G3) or ER+, PR +/-, HER-2+, any Ki67, any PR.

HER-2-positive: ER-, PR-, and HER-2+.

Triple-negative: ER-, PR-, and HER-2-[7].

Focal heterogeneity

Histologic heterogeneity of index intra-foci (HhİİF) (mix type=intra-focal): Heterogeneity of index focus observed in histological type, which is the large tumor size.

Histologic heterogeneity of inter-foci (HhİF): Heterogeneity observed in histological type between foci.

Immunohistochemical heterogeneity of inter-foci (İhİF): Heterogeneity observed in immunohistochemical (phenotypic) subtype between foci.

Statistical analysis

The analyses were carried out using SPSS version 26.0.

RESULTS

The median age of 89 patients included in the study was 48 years. The most dominant findings in the study were as follows: ER+=78.7% (70/89), PR+=73% (65/89), HER-2-=75.3% (67/89), Ki67≥20%=51.7% (46/89), LVI+=61.8% (55/89), G2=65.2% (58/89), two foci=74.2% (66/89), IDC=70.8% (63/89), and homogeneity with the rates of 70.7% (63/89). In total, 29.2% (26/89) of MF/MC breast carcinomas were heterogeneous. Heterogeneity distribution of the relative foci was HhİİF (15.7%, 14/89), HhİF (7.9%, 7/89), and İhİF (10.1%, 9/89). Evaluation of additional foci resulted in alteration of postoperative oncology treatment of three patients according to the histopathology of index focus. In those three (3.3%, 3/89) patients, there was heterogeneity in immunohistochemical subtypes, and the tumor grade was G3 in two patients and G2 in one patient (Tables 1 and 2).

The median duration of clinical follow-up was 83 months (min–max: 5–120 months, mean: 81.3±24 months). Recurrence was recorded in 23.6% (21/89) of the patients during follow-up. The most common postoperative recurrence types were local recurrence (4.5%, 4/89), visceral organ (5.6%, 5/89), bone (12.4%, 11/89), and bone plus visceral organ (1.1%, 1/89). The pathological N (pN) stage and mortality rate were significantly higher (38.1%, 8/21) in the recurrence group as compared to the group without recurrence ($p<0.05$) (Table 1).

The univariate analysis assessing DFS showed that pathological T (pT) stage, pN stage, and histological grade of index focus were significantly effective ($p<0.05$). Similarly, the multivariate analysis indicated that pN stage had a significantly independent (HR=2.29, 95%CI 1.39–3.76, p=0.001) effect on DFS. In the univariate analysis evaluating overall survival (OS), however, pN stage had significant effect only on OS (HR=5.82, 95%CI 1.580–21.43, p=0.008) (Table 3).

DISCUSSION

TNM classification is accepted as the gold standard in breast cancer staging. In its latest version, the largest diameter in MF/MC breast cancers is taken into account, and other foci are not[8]. As a result, cumulative tumor burden does not affect the choice of treatment. In many studies in the literature, it is known that MF/MC breast cancers have higher metastases, recurrence, and decreased survival when compared with similar stage unifocal (UF) breast cancers[6,9-11]. The state of the axillary lymph node (ALN) (or pN stage) is one of the most important prognostic factors. An increase in the number of the state of ALN metastases proportionally decreases DFS and OS. In the literature, the factors indicative of the presence of the state of ALN metastases are large tumor size, presence of lymphovascular invasion, high grade (G3), molecular status, and tumors with lateral or central localization[5,8,12]. This situation has brought along discussions on subjects such as survival, mortality, cost, staging, biological, and

Table 1. Comparison of clinicopathological, recurrent and non-recurrent patients after postoperative oncological treatment.

		Clinicopathological data				Recurrence (-)			Recurrence (+)			p
		Min.–Max.	Median	Mean±SD/n-%		Mean±SD/n-%		Median	Mean±SD/n-%		Median	
		28.0–79.0	48.0	50.4±12.5		50.0±12.5		48.0	53.8±12.9		55.5	0.498 [m]
Age	≤50			50	56.2%	39	57.4%		11	52.4%		0.688 [m]
	>50			39	43.8%	29	42.6%		10	47.6%		
İndex focus T size (cm)		0.3–9.0	2.5	2.9±1.8		2.8±1.8		2.5	3.8±2.2		3.3	0.186[m]
Surgery type	Breast conserving			17	19.1%	16	23.5%		1	4.8%		0.056[x2]
	Mastectomy			72	80.9%	52	76.5%		20	95.2%		
Histologic types of index foci	Ductal carcinoma			63	70.8%	51	75.0%		12	57.1%		0.194[x2]
	Lobular carcinoma			3	3.4%	2	2.9%		1	4.8%		0.558[x2]
	Mix types			14	15.7%	8	11.8%		6	28.6%		0.132[x2]
	Special types			9	10.1%	7	10.3%		2	9.5%		0.755[x2]
Pathological T category	pT1≤2 cm			29	32.6%	24	35.3%		5	23.8%		0.148[x2]
	2 cm<pT2≤5 cm			35	39.3%	28	41.2%		7	33.3%		
	pT3			5	5.6%	4	5.9%		1	4.8%		
	pT4			20	22.5%	12	17.6%		8	38.1%		
Pathological N category	pN0			20	22.5%	20	29.4%		0	0.0%		**0.003[x2]**
	pN1			25	28.1%	19	27.9%		6	28.6%		
	pN2			23	25.8%	18	26.5%		5	23.8%		
	pN3			20	22.5%	10	14.7%		10	47.6%		
	Missing			1	1.1%							
Grade of index foci	I			5	5.6%	5	7.5%		0	0.0%		0.258[x2]
	II			58	65.2%	46	68.7%		12	60.0%		
	III			24	27.0%	16	23.9%		8	40.0%		
	Missing			2	2.2%							
Number of foci	II			66	74.2%	52	76.5%		14	66.7%		0.540[x2]
	III			18	20.2%	12	17.6%		6	28.6%		
	IV			4	4.5%	4	5.9%		0	0.0%		
	V			1	1.1%	0	0.0%		1	4.8%		
İndex focus ER status	≥1%			70	78.7%	55	80.9%		15	71.4%		0.355[x2]
	<1%			19	21.3%	13	19.1%		6	28.6%		
İndex focus PR status	≥1%			65	73.0%	53	77.9%		12	57.1%		0.060[x2]
	<1%			24	27.0%	15	22.1%		9	42.9%		
İndex focus HER2 (SİSH) status	Positive			22	24.7%	18	26.5%		4	19.0%		0.491[x2]
	Negative			67	75.3%	50	73.5%		17	81,0%		
Index focus Ki67 status	≥ 20%			46	51.7%	33	48.5%		13	61.9%		0.284[x2]
	< 20%			43	48.3%	35	51.5%		8	38.1%		
Lymphovascular invasion	Yes			55	61.8%	39	58.2%		16	76.2%		0.137[x2]
	No			33	37.1%	28	41.8%		5	23.8%		
	Missing			1	1.1%							
Perineural invasion	Yes			18	20.2%	14	21.9%		4	20.0%		0.252[x2]
	No			66	74.2%	50	78.1%		16	80.0%		
	Missing			5	5.6%							

Continue...

Table 1. Continuation.

		Clinicopathological data				Recurrence (-)			Recurrence (+)			p
		Min.–Max.	Median	Mean±SD/n-%		Mean±SD/n-%		Median	Mean±SD/n-%		Median	
Perinodal invasion	Yes			39	43.8%	27	40.3%		12	57.1%		0.1752
	No			49	55.1%	40	%59.7%		9	42.9%		
	Missing			1	1.1%							
Histologic heterogeneity of index foci	Homogeneity			75	84.4%	59	86.8%		15	71.4%		0.101 x2
	Heterogeneity			14	15.7%	8	11.8%		6	28.6%		
Histologic heterogeneity inter-foci	Homogeneity			82	92.1%	63	92.6%		19	90.5%		0.888 x2
	Heterogeneity			7	7.9%	5	7.4%		2	9.5%		
Immunohistochemical heterogeneity inter-foci	Homogeneity			80	89.9%	60	88.2%		20	95.2%		0.352 x2
	Heterogeneity			9	10.1%	8	11.8%		1	4.8%		
Follow-up time						84.9±20.6		89.0	45.0±27.7		38.0	0.106 m
Disease-related mortality	No					68	100%		13	61.9%		**0.000 x2**
	Yes					0	0.0%		8	38.1%		
Recurrence	No			68	76.6%							
	Yes			21	23.6%							
Recurrence location	Local			4	4.5%							
	Visceral			5	5.6%							
	Bone			11	12.4%							
	Bone+visceral			1	1.1%							

m Mann–Whitney U test/x2 Chi-square test (Fischer's exact); T: tumor size, N: lymph node; ER: estrogen receptor; PR: progesterone receptor; HER-2: human epithelial growth factor receptor-2; SİSH: Silver-enhanced in situ hybridization; Ki67: proliferation index. Bold indicates statistically significant values.

morphological behaviors in MF/MC breast tumors at a time when personalized surgical and oncological treatments gain momentum.

Breast cancer is a heterogeneous group of neoplasms with clinical, morphological, and immunohistochemical differences within a single tumor or between tumors. Depending on different histological methods employed and the cases selected, 6–75% of these tumors are MC/MF breast tumors. Inter-focal heterogeneity is one of the major discussion topics in MF/MC breast tumors. In the literature, the rates of HhİİF, HhİF, and İhİF in MF/MC breast cancers are reported as 0–17.1, 0–37, 0–12.7%, respectively[2,6,13,14]. Similar to the literature, we found the rates of 15.7, 7.9, and 10.1%, respectively.

In their series dated 2012 of 65 cases with MF/MC IDC, Choi et al. found additional heterogeneous tumor foci that would change the treatment decision in 8% (5/65) of the patients. Choi et al. suggested that immunohistochemical analysis of the index tumor may be sufficient in routine practice, but if there is high-grade, different histological features or heterogeneous ductal carcinoma in situ component in the index tumor, additional foci should be examined[2]. In 2014, Parker et al. evaluated HhİİF (heterogeneity in histological type and grade) and HhİF using three classification systems (i.e., modified Nielsen[15], St. Gallen 2011[16], and Sotiriou[17]] in their study of 100 cases. In the evaluation of additional heterogeneous tumor foci using the modified Nielsen, St. Gallen 2011, and Sotiriou systems, the rate of changes in treatment decision were as follows: 3.63% (4/110), 7.27% (8/118), and 7.27% (8/110), respectively. In MF/MC breast cancers that were classified according to the modified Nielsen system, the risk of death was significantly higher ($p<0.05$) and survival was shorter in the İhİF group. However, there was no significant difference ($p>0.05$) between the groups according to the classifications by the St. Gallen 2011 and Sotiriou classification systems. There was no significant difference ($p>0.05$) between HhİİF MF/MC breast cancers and the other homogeneous group of the series. The authors reported that in patients with inter-focal histological type and/or degree of heterogeneity, up to five foci of inter-focal immunohistochemical evaluation may reduce the risk of death from the disease[6]. Mosbah et al. did not detect any heterogeneity that could change the treatment decision in the study they conducted in 2015 on

Table 2. Heterogeneity distribution of MF/MC breast cancers.

Patient	Number of foci	Size (cm)	Grade of index foci	Histology of intra- index and inter-foci	Histology of inter foci	Immunohistology of inter-foci (St. Galen)	Those whose medical treatment changed
1	2	1.2/0.3	2	D-MP/D-MP	D-MP/D-MP	LuA/LuA	–
2	2	1.9/1.6	3	L-T/L-T	L-T/L-T	LuA/LuA	–
3	2	2.3/2	3	D-MP/D-MP	D-MP/D-MP	LuB/LuB	–
4	2	2.5/1.5	2	M-D/M-D	M-D/M-D	LuB/LuB	–
5	2	3.5/0.2	2	D-M/D-M	D-M/D-M	LuB/LuB	–
6	2	4/2.5	2	D-MP/D-MP	D-MP/D-MP	LuB/LuB	–
7	2	5/0.2	2	N-D/N-D	N-D/N-D	LuB/LuB	–
8	2	2.5/0.3	2	D-MP/D-MP	D-MP/D-MP	HER/HER	–
9	2	5.5/1	2	D-MP/D-MP	D-MP/D-MP	HER/HER	–
10	2	6/0.9	3	M-MP/M-MP	M-MP/M-MP	HER/HER	–
11	3	6/4/1	2	D-L/D-L/D	D-L/D-L/D	LuA/LuA/LuA	–
12	3	3.5/1.9/1.4	3	D-MP/D-MP/D-MP	D-MP/D-MP/D-MP	LuB/LuB/LuB	–
13	2	0.9/0.6	1	C/T	C/T	LuA/LuA	–
14	2	1/0.6	2	D/T	D/T	LuA/LuA	–
15	2	2/0.2	2	C/L	C/L	LuA/LuA	–
16	2	2/1.8	3	D/D-L	D/D-L	Tn/Tn	–
17	3	2.8/2/0.2	2	D/L/L	D/L/L	LuA/LuA/LuA	–
18	2	1.5/1.2	2	D/D	D/D	LuB/LuA	–
19	2	1.8/1.5	2	D-P/D-P	D-P/D-P	LuB/LuA	–
20	2	2.5/2.5	2	D-L/D-L	D-L/D-L	LuB/LuA	–
21	2	4/3	2	D/D	D/D	LuB/LuA	–
22	3	2.4/1.8/1.5	2	D/D/D	D/D/D	LuB/LuB/LuA	–
23	3	2.6/1.1/0.3	2	D/D/D	D/D/D	LuB/LuA/LuA	–
24	2	2.5/2.1	3	D/D	D/D	LuA/LuB	+
25	2	3/1.6	2	D/D	D/D	LuA/LuB	+
26	2	1/1	3	D/D	D/D	Tn/LuB	+

D: invasive ductal carcinoma; L: invasive lobular carcinoma; MP: invasive micropapillary carcinoma; M: mucinous carcinoma; N: neuroendocrine carcinoma; T: invasive tubular carcinoma; C: invasive cribriform carcinoma; LuA: Luminal A; LuB: Luminal B; HER: human epidermal growth factor receptor; Tn: triple negative.

205 cases, in which they performed histopathological evaluations of up to two foci. Mosbah et al. reported that even if there is a discrepancy between tumor foci, it had little effect on the treatment decision, and that even if the focus showed different tumor grades, performing immunohistochemical evaluation on the index tumor focus only may be sufficient[14]. In our study, evaluation of additional foci changed postoperative oncological treatment for three patients. In those three (3.3%, 3/89) patients, the number of foci was two and there was heterogeneity in immunohistochemical subtypes. The histological grade of index tumor foci was G3 in three patients and G2 in one patient (Tables 1 and 2). When compared with the literature considering the cost and individualized oncological treatments, it seems sufficient to evaluate grades 2–3 tumors in the index focus up to two foci. Nevertheless, it should also be noted that heterogeneity (i.e., HhİİF, HhİF, and İhİF) does not have a statistically significant effect on recurrence and survival (DFS and OS) in MF/MC breast cancer (Tables 1 and 3).

Boros et al. evaluated the risk of ALN metastasis in their study of 155 cases with MF/MC breast cancer in 2015 and found that there was not a statistically significant difference between homogeneous patients (73.91%, 85/115) and those (80%, 32/40) with

Table 3. Univariate and multivariate analyses for disease-free survival and overall survival.

Survival	Disease-free survival						Overall survival					
	Univariate model			Multivariate model			Univariate model			Multivariate model		
Disease-free survival	HR	95%CI	p	HR	95%CI	p	HR	95%CI	p	HR	95%CI	p
Age (≤50/>50)	0.99	0.96–1.03	0.628				1.02	0.969–1.08	0.411			
Index focus size (cm)	1.13	0.93–1.37	0.230				1.22	0.912–1.64	0.178			
Surgery type (breast conserving/mastectomy)	4.38	0.59–32.70	0.149				28.28	0.016–>100	0.383			
Histological types of index foci	1.24	0.87–1.79	0.239				1.52	0.891–2.59	0.125			
Pathological T stage	1.58	1.07–2.33	**0.021**				1.82	0.987–3.34	0.055			
Pathological N stage	2.29	1.39–3.76	**0.001**	2.29	1.39–3.76	**0.001**	5.82	1.580–21.43	**0.008**			
Grade of index foci	2.32	1.01–5.32	**0.048**				1.85	0.500–6.85	0.356			
Number of foci	1.52	0.76–3.02	0.236				11.19	0.408–3.47	0.750			
Index focus ER status (ER≥1%/ER<1%)	1.64	0.64–4.23	0.306				2.41	0.575–10.09	0.229			
Index focus PR status (PR≥1%/PR<1%)	2.71	1.14–6.45	**0.024**				2.81	0.701–11.24	0.145			
Index focus HER-2 (SİSH) status (+/–)	1.15	0.39–3.41	0.805				2.21	0.272–17.98	0.458			
Index focus Ki67 status (≥20%/<20%)	0.52	0.21–1.15	0.145				0.61	0.146–2.55	0.499			
Lymphovascular invasion (yes/no)	0.46	0.17–1.27	0.133				0.21	0.025–1.70	0.143			
Perineural invasion (yes/no)	1.36	0.45–4.07	0.586				1.85	2.223–15.46	0.568			
Perinodal invasion (yes/no)	0.67	0.28–1.58	0.356				0.24	0.049–1.21	0.084			
Histologic heterogeneity of index foci (homogeneity/heterogeneity)	1.90	0.73–4.89	0.186				3.47	0.823–14.60	0.090			
Histologic heterogeneity of inter-foci (homogeneity/heterogeneity)	1.10	0.26–4.74	0.895				1.71	0.210–13.93	0.616			
Immunohistochemical heterogeneity of inter-foci (homogeneity/heterogeneity)	0.32	0.04–2.39	0.267				0.04	0.000–984.83	0.537			

Cox regression (forward LR); T: tumor size; N: lymph node; ER: estrogen receptor; PR: progesterone receptor; HER-2: human epithelial growth factor receptor-2; SİSH: Silver-enhanced in situ hybridization; Ki67: proliferation index. Bold indicates statistically significant values.

mismatches between histological type, grade, or both regarding the rate of ALN metastases (p>0.05). The rate of heterogeneous MF/MC breast cancer determined ALN metastases as follows: 72.73% (8/11) with histological type heterogeneity, 87.5% (14/16) with inter-focal grade heterogeneity, and 76.92% (10/13) with histological type and grade heterogeneity. Most (33.33%, 8/24) of the heterogeneous MF/MC tumors with differences in inter-focal grade (87.5%, 21/24) had a high histological grade. In that study, the authors stated that the histological features (e.g., type and grade) of ALN metastases in MF/MC breast cancers correspond to the histological type with unfavorable prognosis or the highest histological grade, which is not necessarily of the index focus, and emphasized that it is necessary to conduct an individual histopathological evaluation for each tumor focus with high grade[5]. It is known that before the inclusion of prognostic biomarkers (e.g., grade, hormone receptor, oncogene expression,

and multigene panel recurrence score) in the current TNM classification, hormonotherapy increased the long-term survival by decreasing the recurrence rate of ER+/PR+ breast tumors, yet had no effect on long-term survival in ER+/PR- breast tumors[8,18]. In our study, a significant ($p<0.05$) effect of pT stage, pN stage, and index focus histological grade was observed in univariate analysis in which DFS was evaluated. In multivariate analysis, pN stage had a significantly independent (HR=2.29, 95%CI 1.39–3.76, p=0.001) effect on DFS. In the univariate analysis evaluating OS, however, pN stage had a significant effect only on OS (HR=5.82, 95%CI 1.580–21.43, p=0.008) (Table 3). This result supports the literature.

The limitations of the study are as follows: it evaluated the long-term effects, it had a retrospective design, and it could not evaluate the histological/immunohistochemical footprints of the foci in metastatic ALN.

CONCLUSION

At present, when oncological treatments are personalized with a multidisciplinary approach, less than 4% of patients have heterogeneity in MF/MC breast cancers, which necessitate an alteration in therapeutic decisions. As evaluating multiple foci would increase the cost and labor, it may be enough to evaluate grade 2–3 tumors in the index focus up to two foci. However, heterogeneity does not have a significant effect on recurrence and survival in MF/MC breast cancers. The pN is an independent risk factor for DFS.

ETHICAL APPROVAL

All procedures performed in this study, involving human participants, were in accordance with the ethical standards of the İnstitutional Research Committee and 1964 Helsinki Declaration. Local ethics committee approval was obtained from Health Sciences University, Istanbul Training and Research Hospital (dated November 13, 2020, Decision no.: 2588).

AVAILABILITY OF DATA

All data generated or analyzed during this study are included in this published article (and its supplementary information files). But restrictions apply to the availability of these data, which were used under license for the current study, and so are not publicly available. Data are, however, available from the authors upon reasonable request and with the permission of Health Sciences University Turkish Ministry of Health İstanbul Research and Training Hospital.

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AUTHORS' CONTRIBUTIONS

FD: Conceptualization, Data curation, Formal Analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, and Writing – review & editing. **HÖ:** Conceptualization, Data curation, Formal Analysis, Investigation, Resources, Software, Supervision, Validation, and Visualization. **KU:** Conceptualization, Data curation, Formal Analysis, Investigation, Resources, Software, Supervision, Validation, and Visualization. **ŞÇ:** Data curation, Formal Analysis, Investigation, Methodology, Resources, Software, Supervision, Validation, and Visualization. **SBH:** Data curation, Formal Analysis, Investigation, Methodology, Resources, Software, Supervision, Validation, and Visualization. **FDCT:** Data curation, Formal Analysis, Investigation, Methodology, Resources, Software, Supervision, Validation, and Visualization. **EF:** Data curation, Formal Analysis, Investigation, Methodology, Resources, Software, Supervision, Validation, and Visualization. **SS:** Methodology, Resources, Supervision, Validation, Visualization, and Writing – review & editing.

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ORIGINAL ARTICLE

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Frequency of Congenital Aortic Arch Anomaly in COVID-19 Patients

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SUMMARY

OBJECTIVE: The aim of this study was to investigate the frequency of aortic arch anomaly in COVID-19 patients and to determine whether it will be included in the risk classification.

METHODS: The study was retrospectively conducted in a third-level hospital by scanning the contrast-enhanced thoracic computed tomography and thoracic computed tomography angiography examinations of patients who received PCR (+), hospitalization, and known COVID pneumonia between March 2020 and July 2021. The study consists of 88 cases and 88 control groups.

RESULTS: The study found that the frequency of aortic arch anomaly was higher in patients with COVID-19 pneumonia and in male patients with bovine-type anomaly.

CONCLUSIONS: The higher prevalence of bovine arch anomaly in COVID patients may be considered a risk factor for COVID-19 in individuals with this type of vascular anomaly.

KEYWORDS: COVID-19. CT angiography. Arch of the aorta.

INTRODUCTION

COVID-19 is a highly contagious disease caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), which is an infective strain. This virus is in the RNA genome structure, and it has been causing a pandemic all over the world since December 2019 and can cause a wide range of symptoms from mild to moderate signs such as dry cough, fever, and myalgia to severe clinical signs such as acute respiratory distress syndrome (ARDS)[1]. In addition, it is found that it also causes serious symptoms on the brain, heart, and liver[2]. As of November 2021, there have been 247 million cases and 5 million death reports worldwide (https://covid19.who.int/).

The following diseases are included in the content definition of chronic risk groups for COVID-19[3,4]:

- Advanced age (>65 years)
- Receiving immunosuppressive therapy (e.g., HIV, long-term steroid therapy)
- Metastatic cancer patients or receiving chemotherapy/radiotherapy
- Those with solid organ transplants, excluding cornea transplant
- Patients with bone marrow/stem cell transplantation
- Those with lung diseases such as chronic obstructive pulmonary disease (COPD), lung cancer, cystic fibrosis, pulmonary fibrosis, and moderate-to-severe asthma patients
- Insulin-dependent diabetes and its complications (i.e., cerebrovascular, coronary, renal, and polyneuropathy)
- Patients with non-insulin-dependent diabetes mellitus
- Patients with complicated hypertension (i.e., cerebrovascular, renal, and congestive heart failure)
- Cardiac patients with cardiomyopathy, pulmonary hypertension, congenital heart diseases, heart failure, and coronary artery disease
- Patients with chronic liver disease or chronic renal failure
- Patients with cerebrovascular disease (e.g., stroke and bleeding)
- Patients with blood cell disease (e.g., sickle cell anemia and thalassemia patients)
- Down's syndrome

Thoracic vascular anomalies

Congenital thoracic vascular anomalies consist of a large group, which includes the thoracic aorta and its branches, pulmonary arteries, thoracic systemic veins, and pulmonary veins[5]. These anomalies can be either accompanied by congenital heart diseases or seen alone. These anomalies can be completely asymptomatic or they can create esophageal, take

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pressure effect, and create clinics that may be related to cardiovascular, respiratory, and nutrition[6].

No identification of the COVID-19 disease risk group has been made for congenital vascular anomalies and variations. In our study, the types and frequency of vascular anomalies of the arcus aorta in COVID-19 patients were investigated and predictions were made for the risk group.

Anomaly of the aortic arch and its variants

This group includes the left aortic arch, the right aortic arch, the double aortic arch, and the large vessel branching patterns[7]. The left aortic arch continues as the descending aorta, which crosses the left main bronchus at the level of the T5 vertebral corpus and descends to the left of the midline. Its incidence is found to be between 70 and 80%[7]. Among the large vessel anomalies branching from the left aortic arch, the most common is the bovine type of arcus anomaly. The frequency of bovine-type arch anomalies in the general population varies between 9 and 13%. An anomaly of the left vertebral artery of the aortic arch origin is observed in 5–6% of the population. Another frequent anomaly is the aberrant right subclavian artery anomaly with the left aortic arch, which is observed in 0.5–2% of the population[7].

Imaging methods have a very important place in the detection of these anomalies and in the preoperative evaluation[8]. Due to the advancement of technology, contrast-enhanced computed tomography (CT)/CT angiography and non-contrast-enhanced magnetic resonance imaging (MRI) examinations are now primarily selected from noninvasive methods. With these examinations, more detailed and three-dimensional images can be obtained[8].

Limitations

The study group may not fully reflect the general population due to the fact that there are inpatients diagnosed with COVID-19 and the contrast-enhanced thoracic CT and thoracic CT angiography examinations performed as an examination.

METHODS

This study was conducted in a third-line hospital, retrospectively, between March 2020 and July 2021, among 88 patients aged 18–90 years, who received PCR (+), inpatient treatment, and with known COVID pneumonia, and randomly selected 88 patients without a diagnosis of COVID-19 as control group. Ethics committee approval of the study was obtained with protocol number GOKA/2021/11/8. In the study, contrast-enhanced thoracic CT and thoracic CT angiography examinations were examined in both the patient and control groups. After contrast-enhanced thoracic CT examinations, contrast media, 5 mm thick sections were taken, and after 2.5 mm reconstructions, images were obtained in the lung parenchyma and mediastinal Windows. Thoracic CT angiography examinations were taken for thoracic structures after injection of contrast medium together with the test bolus, and images were obtained with 5 mm thick sections and 1 mm reconstructions.

Statistical analysis

SPSS version 21.0 package program was used for statistical analysis. Descriptive statistics are expressed as mean±standard deviation for continuous data and number and percentage distributions (%) for frequency data. The Shapiro-Wilk test was used to evaluate whether the data met the parametric conditions. Chi-square test was used in the comparison of categorical variables. Student's t-test for pairwise comparisons of independent groups and one-way analysis of variance (ANOVA) for comparisons of more than two groups were used to determine the difference between groups. In the evaluations, $p<0.05$ was accepted as the statistical significance level for all tests.

RESULTS

The study consists of a total of 176 patients, including 88 cases and 88 control groups. The mean age of the case group was 61, the mean age of the control group was 59, and the demographic (e.g., age and gender) data of the study are given in Table 1.

The frequency of vascular anomalies was found to be 34% (30) in the case group. Of these, 27 (31%) bovine arcus aortic anomalies and 3 (3%) other classified as vascular anomalies (2 left vertebral artery anomalies of aortic origin, 1 right aberrant subclavian artery anomaly) were detected. In the case group, 58 (66%) patients without vascular anomalies were found (Table 2).

The frequency of vascular anomalies in the control group was 21% (19). Of these, 17 (19%) bovine-type aortic arch anomalies and 2 (2%) classified as other types (2 left vertebral artery anomalies originating from the aorta) were found (Table 2). Although the frequency of vascular anomalies was higher in the case group, no statistically significant difference was observed ($p>0.05$).

Table 1. Demographic data.

	Case group		Control group	
	Average age	Count	Average age	Count
Male	60±14	60	58±13	52
Female	65±15	28	60±12	36
Total		88		88

Table 2. Vascular anomaly frequency distribution.

	Case group	Control group
Normal	58	61
Bovine-type archus anomaly	27	17
Anomaly of the left vertebral artery of aortic origin	2	2
Anomaly of the right aberrant subclavian artery	1	0

Table 3. Frequency distribution of vascular anomalies by gender.

	Case group		Control group	
	Male	Female	Male	Female
Bovine-type archus anomaly	22	19	10	7
Anomaly of the left vertebral artery of aortic origin	2	0	0	2
Anomaly of the right aberrant subclavian artery	1	0	0	0

When the vascular anomaly types and gender were compared in the case group, the incidence of bovine-type anomalies was found to be 81% (22) in men and 19% (5) in women, showing male dominance to be statistically significant (p=0.02). In the classification as other type, all three cases were found to be male gender (Table 3).

When the vascular anomaly types and gender were compared in the control group, the frequency of bovine-type anomaly was 60% (10) in men and 40% (7) in women. In the classification made as other type, all two cases were found to be female and no statistically significant difference was observed between the genders (p>0.05) (Table 3).

In the case group, the average age of those with vascular anomalies was 64±14, and in control group it was 60±15, which is more advanced, but not statistically significant (p>0.05).

DISCUSSION

It is known that since the COVID-19 disease has been identified, patients with various risk factors are more likely to develop this disease and have a predisposing effect for COVID-19 pneumonia[3,4].

According to the VASCERN (European reference network for rare vascular diseases) vascular anomaly study group, there is no consensus on the risk of COVID-19 disease for people with vascular abnormalities; however, it has been stated that vascular anomalies that cause the heart, lungs, kidney, and liver to be affected may pose a high risk. For this reason, the occurrence of severe and severe effects of COVID-19 in people with complicated vascular anomalies caused by cardiac and lung diseases has been considered a high risk[9]. Microdeletions were found in *22q11.2* gene in thoracic midline anomalies, such as thymic hypoplasia, DiGeorge syndrome, conotruncal anomalies, truncus arteriosus, and tetralogy of Fallot. About 25–35% of thoracic arch anomalies have been found to have microdeletions in *22q11.2* gene, even without congenital cardiac defect[5,7]. This shows that there are some genetic defects in isolated arch anomalies. Likewise, the genetic basis of which has not yet been clarified, but also in COVID-19 patients, some mutations are also a factor in catching the disease and having a severe disease, which makes us think that it is effective in its reflection in the clinic.

In our study, the incidence of thoracic arch vascular anomalies is more common in COVID-19 patients, and the incidence of bovine type of arcus aortic anomaly is also higher in them (31%). According to Hanneman et al. and Priya et al., the normal prevalence of the bovine-type arch anomaly varies between 9 and 20%[7,8,10]. The higher prevalence of bovine-type arch anomaly in COVID patients can be considered a risk factor for COVID-19 disease in individuals with this type of vascular anomaly.

The frequency of bovine-type arch anomaly was higher in male gender COVID-19 patients. According to Jeffrey et al., more arc anomalies were detected in the male sex and are compatible with our study[5].

In our study, the prevalence and gender distribution of vascular arch anomalies, which were classified into "other" and seen less frequently, were similar to the general population[7,8].

CONCLUSION

As a result, it can be said that the incidence of thoracic arch anomalies is higher in COVID-19 patients. The fact that the population can be made with new studies to include larger patient groups and that additional imaging tests that do not cause radiation exposure, such as echocardiography, can be added to provide more objective data.

ETHICS APPROVAL

The study was obtained with protocol number GOKA/2021/11/8.

AUTHORS' CONTRIBUTIONS

MMA: Conceptualization, Data curation, Formal Analysis, Investigation, Methodology, Project administration, Writing – original draft. **MY:** Data curation, Investigation, Supervision, Validation, Resources.

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REVIEW ARTICLE

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Management of ultrasonographic endometrial thickness in postmenopausal asymptomatic women

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INTRODUCTION

Endometrial cancer is the sixth most diagnosed type of cancer in women worldwide[1]. In 90% of patients, it is associated with transvaginal bleeding. Its occurrence is low in menacme. At menopause, the endometrium should be investigated when transvaginal bleeding occurs[2].

The incidental finding of thickened endometrium in postmenopausal women is common, and the cutoff thresholds for investigating the changes in women with transvaginal bleeding are well established. However, there is no consensus in asymptomatic patients[3]. Patients with postmenopausal transvaginal bleeding and endometrial thickness above 4–5 mm have universally accepted cutoff values for cancer investigation[4].

The present study aims to evaluate the need for endometrial investigation with invasive methods of incidental ultrasonographic findings of endometrial thickening in postmenopausal patients without transvaginal bleeding, avoiding unnecessary patient risks, such as perforations, physical and psychological trauma, as well as expenses for health services.

METHODS

Registration

This study comprised a systematic review of articles and was registered with the PROSPERO registry for systematic reviews (ID CRD42022297524).

Study selection

The selection of articles was performed by three independent evaluators, according to the inclusion and exclusion criteria. Articles were initially evaluated by title. Subsequently, the articles were evaluated by abstract. Differences were resolved by consensus among researchers. This method was used to achieve the appropriate methodological quality of the systematic review.

Information sources and search

This systematic revision was based on a review of the literature in the PubMed databases, between 2010 and 2020. The MeSH (Medical Subject Headings) terms used were postmenopause, ultrasonography, systematic revision, and meta-analysis. Endometrial thickness was also a keyword used. Exclusion criteria were articles published before 2010 and publications that were not in the English language. A total of 95 articles were found. After reading the titles of the articles, it was noticed that some of them did not fulfill the criteria of this study. A total of 14 articles were selected for reading the abstract and those that did not relate to the purpose of this study were excluded (Figure 1). Table 1 shows the analyzed studies.

ENDOMETRIAL CANCER AND ULTRASONOGRAPHIC ENDOMETRIAL THICKNESS

The accuracy of ultrasonography addressing gynecology is evaluated in some studies[5,6]. Transvaginal ultrasound is an important examination for the investigation of endometrial cancer in patients with postmenopausal bleeding. However, in asymptomatic patients, there is no consensus on when to investigate. In patients with postmenopausal bleeding, endometrial thickening is recommended and most studies suggest further investigation with endometrial biopsy when they present values greater than 4 or 5 mm on transvaginal ultrasound imaging. In 10% of cancer cases, patients may be asymptomatic[7,8] and are suspected after an annual routine transvaginal ultrasound[9].

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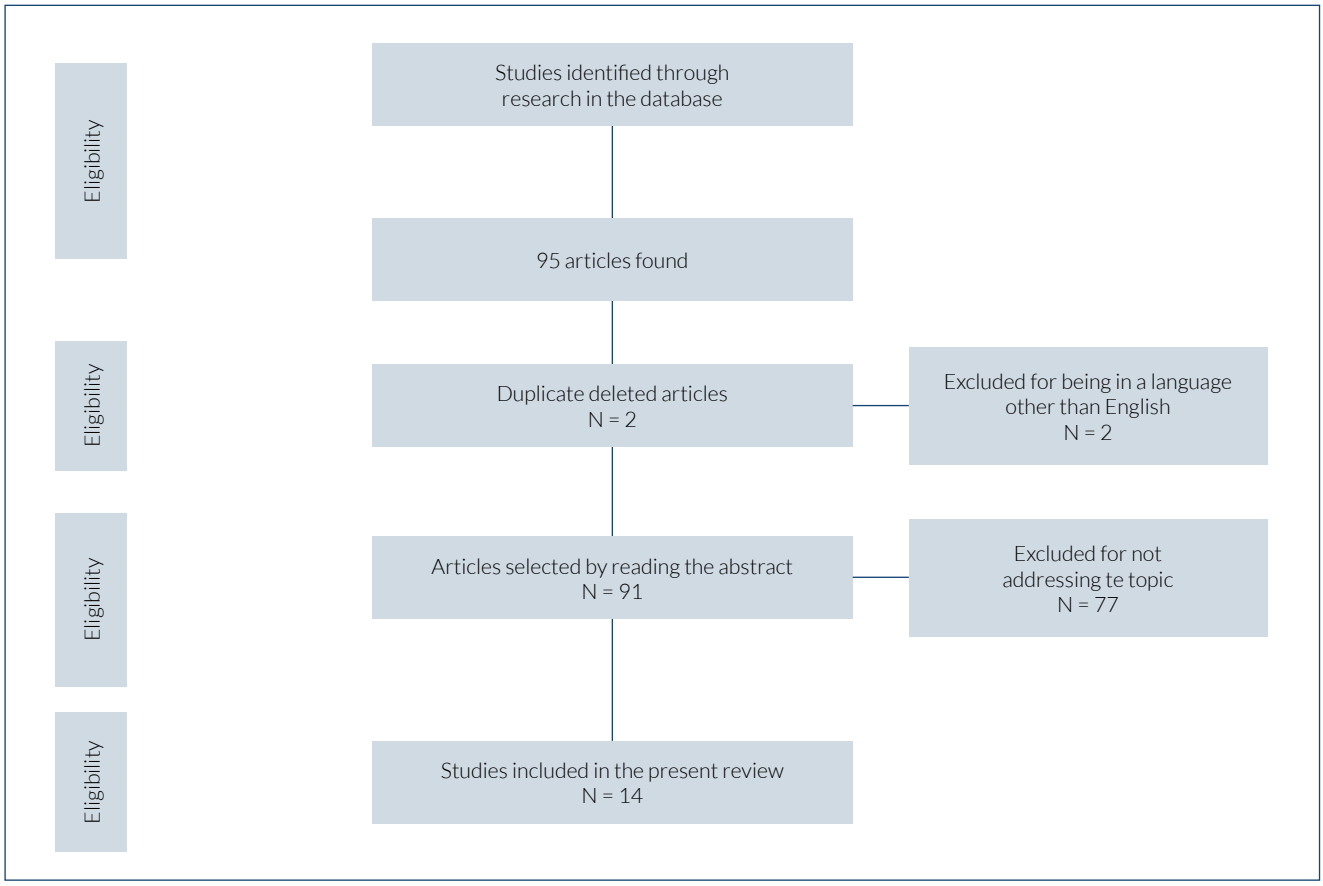

Figure 1. Study flowchart.

Despite increasing relatively the chances of an early diagnosis, ultrasound should not be used as a screening method[10-12], as they may increase the risk of complications[13].

Goldstein et al.[13] demonstrated that the incidence of cancer in asymptomatic patients with polyp images at ultrasound is approximately 0.4%. Therefore, there was no therapeutic advantage in investigating these patients in the absence of transvaginal bleeding, except in patients at high risk for endometrial hyperplasia, polycystic ovary syndrome, metabolic syndrome, diabetes, hypertension, or obesity[13].

A study evaluated 65 patients without transvaginal bleeding in the postmenopausal period. Of these, 11 (16.9%) patients had leiomyomas, 28 (43.0%) had endometrial polyps, 0 had adenocarcinomas, 2 (0.03%) had atypical hyperplasias, and 4 (0.06%) had simple or complex hyperplasia. This study concluded that the value of 5 mm is too small to be investigated in the cases of absence of postmenopausal bleeding, which did not identify any patient with endometrial cancer in asymptomatic patients, with an average endometrial thickening of 9.7 mm[14].

Another study evaluated 259 postmenopausal patients without uterine bleeding. All patients were submitted to transvaginal ultrasound and hysteroscopy. There were 214 (82.6%) patients with atrophic endometrium, 20 (0.07%) with simple hyperplasia, 15 (0.05%) with endometrial polyps, 9 (0.03%) with atypical hyperplasia, and 1 (0.003%) with endometrial cancer. The accuracy of the transvaginal ultrasound was analyzed to detect changes in different cutoff values. It was observed that with 3 mm, there is 84.4% sensitivity and 64.4% specificity and with 9 mm, the sensitivity decreased (26.6%) and specificity increased (99.1%). The study compared the effectiveness of transvaginal ultrasonography in detecting intrauterine lesions compared with hysteroscopy and biopsy. Only one endometrial cancer was found, and the risk of cancer in the endometrium <5 mm is 0.07%, while in the endometrium >5 mm it becomes 7.3%. The value of 11 mm separates patients at low risk of cancer from those at high risk of cancer. According to this study, ultrasonography should be used to screen for intrauterine pathologies in asymptomatic patients and has moderate accuracy[2].

In contrast to the above study, a study concluded that transvaginal ultrasound should not be used routinely in asymptomatic patients. In the study, 1,500 women underwent

Table 1. Results of the studies.

Reference		Author	Year	Main results
1	13	Goldstein et al.	2011	The authors demonstrated that the incidence of cancer in asymptomatic patients with polyp images at ultrasound is approximately 0.4%.
2	14	Worley et al.	2011	The study concluded that the 5 mm value is too thin to be investigated in cases of absence of postmenopausal bleeding.
3	2	Kasraesian et al.	2011	Ultrasonography should be used to screen for intrauterine pathologies in asymptomatic patients and has moderate accuracy.
4	15	Hartman et al.	2013	A transvaginal ultrasound should not be used routinely in asymptomatic patients.
5	16	Gianella	2014	The majority of women had an endometrium between 4 and 7 mm, and none of these had pre-malignant or malignant lesions.
6	11	Aston and Weaver	2014	Investigating asymptomatic women provides relatively rare cancer findings and does not improve the patient's prognosis when compared to investigations initiated within 8 weeks of the first transvaginal bleeding.
7	17	Saatli et al.	2014	The study performed a review of medical records in a service in which routine transvaginal ultrasounds are performed for all patients.
8	18	Korkmazer et al.	2014	Transvaginal ultrasound examination was offered to all patients, and a 5 mm cutoff point was used for the possible presence of intrauterine pathology.
9	4	Layemo et al.	2015	The study concluded that if the cutoff value of 11 mm were adopted, 41 patients would not have undergone unnecessary hysteroscopy.
10	19	Louie et al.	2015	The study concluded that 11 mm would be an optimal cutoff point.
11	20	Yasa et al.	2016	The study indicated low accuracy between transvaginal ultrasounds.
12	10	Ozelci et al.	2019	Considering pre-malignant and malignant lesions, the ideal cutoff was 10.5 mm.
13	21	Ghoubara et al.	2018	Patients with an endometrium greater than 4 mm and without bleeding were included.
14	22	Alcázar et al.	2018	The study concluded that patients with an endometrium ≥11 mm have a risk of 2.59 times greater than patients with an endometrium between 5 and 10 mm.

transvaginal ultrasound; 77.1% had an endometrium <4 mm and 92% had <5 mm. In 101 (6.7%) women, a polyp was suspected using ultrasound imaging. Of these women, 97 of 101 (96%) had an endometrium >4 mm, and 89 of 101 (88%) had an endometrium >5 mm. The study concluded that transvaginal ultrasonography is effective for population screening due to its high negative predictive value in patients with postmenopausal transvaginal bleeding; however, it should not be used routinely for asymptomatic women due to its low positive predictive value. In addition, postmenopausal bleeding is an early predictive symptom consistent with endometrial cancer[15].

Gianella et al.[16] conducted a study on 268 asymptomatic patients with an endometrium ≥4 mm. Endometrial biopsy showed 156 (56.8%) atrophies, 92 (34.4%) polyps, 12 (4.5%) submucosal myomas, 8 (2.9%) endometrial hyperplasia, and 4 (1.4%) adenocarcinomas. The best cutoff value was 8 mm (sensitivity 79.3% and specificity 92.1%). This value would reduce the percentage of unnecessary hysteroscopies by 37.4% while recognizing any premalignant or malignant lesions. The highest cutoff value for not failing to recognize a case of endometrial cancer was 10 mm. This study observed that the majority of women (61.2%) had an endometrium between 4 and 7 mm and none of them had premalignant or malignant lesions, concluding that all hysteroscopies were unnecessary in these cases[16].

Aston and Weaver[11] reviewed medical records of women who underwent hysteroscopy, dilation, and curettage. The sample ranged from 5 to 18.1 mm (average of 9.01 mm), and one case of endometrial cancer was identified. In addition, there were four unwanted outcomes (11.4%), including uterine perforation, severe laryngospasm, and severe post-procedure bleeding. The study found that investigating asymptomatic women provides relatively rare cancer findings and does not improve the patient's prognosis when compared to investigations initiated within 8 weeks of the first transvaginal bleeding[11].

Saatli et al.[17] also performed a review of medical records in a service in which routine transvaginal ultrasounds are performed for all patients. The study found five cases of endometrial adenocarcinoma and concluded that one case of cancer is detected only for every 106 investigations carried out using this method[17].

In the study by Korkmazer et al.[18], 197 patients were evaluated by hysteroscopy and histopathological examination. Transvaginal ultrasound examination was offered to all patients, and a 5 mm cutoff point was used for the possible presence of intrauterine pathology. The hysteroscopic findings revealed that 74 (37.7%) patients had normal uterine cavities, 30 (15.2%) had endometrial hyperplasias, 17 (8.6%) had submucosal fibroids, and 76 (38.5%) had endometrial polyps. No malignant or premalignant lesions were seen below 9 mm[18].

Laiyemo et al.[4] evaluated endometrium between 2.8 and 40 mm (average 10.38 mm). Of the 22 cases who had the value above 11 mm, 2 were malignant (21 and 27 mm), and of those who had the value below 11 mm, no cases of malignancy were detected. The risk above 11 mm was 9.1%. Thus, 61 (92.42%) cases were benign. The study concluded that if the cutoff value of 11 mm were adopted, 41 patients would not have undergone unnecessary hysteroscopy[4].

Louie et al.[19] carried out a retrospective cohort study to obtain an optimum cutoff point for biopsy in ultrasound findings of endometrial thickening above 4 mm. A total of 462 patients were analyzed and 435 had alterations (192 patients had endometrial polyps, 18 had simple hyperplasias, 7 cases had hyperplasia with atypia, and 9 had endometrial carcinoma). In this study, the values above 14 mm were associated with atypical hyperplasia and the value of 15 mm was associated with endometrial carcinoma. The study concluded that the value of 11 mm would be an optimal cutoff point[19].

Yasa et al.[20] evaluated 276 asymptomatic postmenopausal patients undergoing curettage or hysteroscopy. If the cutoff value of endometrium was 4 mm, the results were as follows: 107 (38.8%) patients had polyps, 42 (15.2%) had atrophies, 39 (14.1%) had exposure to estrogen, 19 (6.9%) had normal endometrium, 9 (3.3%) had atypical hyperplasias, 8 were diagnosed of endometrial cancer (2.9%), and 52 had insufficient samples (18.8%). The values between 4 and 7 mm revealed that 83 of 89 cases had benign evaluation; between 8 and 11 mm, 69 of 74 cases were normal; and above 12 mm, 55 of 66 did not present any changes. The study indicated low accuracy between transvaginal ultrasound and carcinoma in these conditions[20].

In the study by Ozelci et al.[10], 266 postmenopausal patients without bleeding with endometrium greater than 6 mm underwent hysteroscopy with biopsy, of which 168 (63.1%) patients had polyps, 24 (9%) had simple hyperplasia, 4 (1%) had atypical hyperplasia, and 8 (3%) had endometrial cancer. Of the total number of patients evaluated, 152 had an endometrium between 6 and 10 mm. The optimal cutoff value was 13.5 mm for malignant lesions and atypical hyperplasia (58% sensitivity and 75% specificity). Considering premalignant and malignant lesions, the ideal cutoff value was 10.5 mm (77% sensitivity and 62% specificity)[10].

In another study, 81 patients with an endometrium greater than 4 mm and without bleeding were included. If the cutoff value was 4 mm, the results obtained were as follows: 77 cases had normal endometrium, 57 cases had benign findings, 20 cases had polyps, and 4 cases had hyperplasia or cancer. If the cutoff value was <10 mm, 42 cases were normal, 4 cases had polyps, and no cases had cancer. When the cutoff value was ≥10 mm, 15 cases were normal, 16 had polyps, and 4 cases had atypical hyperplasia or cancer[21].

Alcázar et al.[22] concluded that patients with an endometrium ≥11 mm have a risk of 2.59 times greater than patients with an endometrium between 5 and 10 mm[22].

The limitations of the systematic review are limited to 10 years and the subject use of English-written manuscripts. The inclusion of articles in the past 10 years can be justified by looking for more up-to-date information. A search for articles in English is limited to the subject of Portuguese literature.

CONCLUSION

There is no consensus on the ideal cutoff value for the investigation of endometrial thickening in asymptomatic postmenopausal patients, but it is clear that the value of 5 mm, used in patients with postmenopausal bleeding, increases the number of unnecessary investigations. Transvaginal ultrasonography is of great value in screening for endometrial pathologies in women with postmenopausal bleeding, but it should not be used in routine asymptomatic women.

ACKNOWLEDGMENT

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AUTHORS' CONTRIBUTIONS

LRC: Data curation, Methodology, Writing – original draft. **MPJ:** Investigation, Methodology, Writing – original draft. **MKOG:** Data curation, Methodology, Writing – original draft. **EFCM:** Conceptualization, Formal Analysis, Supervision, Validation, Writing – review & editing. **RSN:** Conceptualization, Data curation, Formal Analysis, Investigation, Methodology, Project administration, Writing – review & editing, Guarantor.

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REVIEW ARTICLE

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Civil sentencing in health care and their relation with blanket consent in rulings from the Court of Appeals of the State of São Paulo

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INTRODUCTION

As of recently, decisions by appellate court chambers in the São Paulo Court of Appeals have been based on a special appeal judged by the Superior Court of Appeals, Special Appeal no. 1.540.580-5/DF[1], 4th Panel. Presiding Justice Lázaro Guimarães, Invitee Justice of the TRF 5th Region, tried on August 2, 2018.

This seems to introduce a further concern for health care professionals who are concerned with detailed patient consent. Even though some form of consent exists, the Justices may consider it null, through the decree of it being a generic consent, also called blanket consent.

OBJECTIVE

The aim of this study was to study legal cases in which there was a condemnatory decision and to verify what was common among the studied processes. In all of them, there was mention of the Special Appeal no. 1.540.580-5/DF[1].

METHOD

The research was conducted in the Case Law section of the website www.tjsp.jus.br, using the term blanket consent only. Therefore, the Court's search tool brought up 17 cases tried and subject to *res judicata*. The research was conducted in the second week of November 2021 by the authors themselves. As a primary requirement of inclusion, the decisions should have the express indication of the Special Appeal no. 1.540.580-5/DF[1]. Conditions for the inclusion of the decisions in the research were as follows: (a) the consent form should be present and should be in written — cases in which there was a vocal explanation by the health professionals or lack of information were excluded; (b) the expert evidence should be favorable to the health professionals; and (c) there should be a civil condemnation of the health professionals.

After applying these additional inclusion filters on the 17 processes found, we analyzed 5 cases that passed the inclusive criteria.

RESULTS

Case 1

The plaintiff underwent a 24-h Holter monitor. When the device was removed, she noticed spots on her skin, which made her students mock her because she is a public school teacher. Expert examination ruled out malpractice in the Holter monitor. The high court Justices' decision was based on the lack of information at the time of the initial appointment since the defendant's clinic only provided a leaflet containing generic information about the use of the device. Such leaflet was deemed invalid since it did not show the plaintiff's acceptance, based on the lack of signature.

Appellate Court conviction: BRL 5,000.00 for punitive damages.

Transcript of decision excerpt

"In this case, the defendant does not even refute that she did not present a signed document in the case records proving that the duty to provide information was required, there was no evidence of any information that should be provided to the plaintiff, whether on the possibility of formation of noticeable scars, appearance of skin spots, skin discoloration or pigmentation in the area where the electrodes are fixed, among others, hence,

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an unequivocal failure to comply with the duty to provide information." (Civil Appeal no. 1014524-26.2019.8.26.0161)[2]

Case 2

Plaintiff underwent a surgery for the removal of one fallopian tube. Nonetheless, during surgery, both fallopian tubes were removed. The second tube was healthy. The plaintiff has not previously consented to any intervention in the second tube. The doctor has an inexorable duty to inform. Witnesses converged on the correctness of the surgical procedure.

Appellate Court conviction: BRL 10,000.00 for punitive damages.

Transcript of the decision's excerpt

"What can be concluded, from all the elements compiled, is that, although correct, the procedure for removing the healthy tube was carried out without the prior and essential consent of the Plaintiff. In this case, the Plaintiff was fully aware that one of her tubes would be removed, due to a previously diagnosed ectopic pregnancy, however, she did not know that the other, healthy, would also be removed, which constitutes a violation of the duty to inform, one of the most relevant in the doctor-patient relationship." (Civil Appeal no. 1017092-69.2017.8.26.0004)[3]

Case 3

Plaintiff underwent plastic surgery on the eyelids and breasts for hypertrophic scars and breast asymmetry. Although the expert report indicated correctness in the surgical procedure, the judges understood that the plaintiff was not clear about the risks, dangers, and disadvantages that the procedures could cause. Two surgeries were performed with an attempt to correct the aesthetics of the breasts, but the plaintiff died from other causes unrelated to the consequences of the surgery.

Appellate Court conviction: BRL 25,000.00 for punitive damages.

Transcript of the decision's excerpt

"As can be seen from the medical documents attached to the case records, and in accordance with a medical expert report, the Plaintiff, on 12.16.2013, aiming at the correction of expression signs around the mouth and correction in the lower and upper eyelids, performed the surgical procedure called 'rhytidectomy' (pages 43 and 221 entry record; pages 223/224 medical record), in addition to being also submitted to the exchange of breast implants on 02.11.2014 (pages 43). It is undisputed, therefore, that the interventions to which the Plaintiff was submitted had a single and exclusive plastic purpose, aiming at the legitimate expectation of obtaining a beautifying result and improving her appearance, so that the obligation assumed by the defendant plastic surgeon was characterized as an obligation of achieving an specific result. Indeed, according to the expert report produced, the facial surgery of rhytidectomy to which the Plaintiff was submitted is not risk free, as well as the complications alleged by the Plaintiff in breast surgery are likely to occur. Therefore, the lack of information by the doctor to the patient is considered to be contractually illegal, configuring the professional's malpractice in the form of negligence (omission in the duty to inform), giving rise to compensation for punitive damages." (Civil Appeal no. 0002255-87.2015.8.26.0653)[4]

Case 4

Plaintiff underwent arthroplasty for placement of a total knee prosthesis. Postoperative infection caused the plaintiff's death. The expert evidence concluded that diabetes and the plaintiff's advanced age were complicating factors for the recovery after surgery, and due to the pathologies, they motivated the serious infection. The plaintiff passed away. Although the expert report was favorable to the doctors, the judges understood that there was a failure in the duty to provide the proper information, including the treatment of infections and partial limb amputation.

Appellate Court conviction: BRL 40,000.00 for punitive damages.

Transcript of the decision's excerpt

"Subsequently, he signed an 'Informed Consent Form for Surgical Procedure' (pages 2417), written in the following terms:

'(...) 2) I received all the necessary information regarding the risks, benefits, treatment alternatives, as well as I was informed about the risks and benefits of not taking any therapeutic action regarding the nature of the diagnosed disease(s).

3) I understand that during exams and/or procedures: _____, in an attempt to cure or improve the aforementioned condition(s), unpredictable or fortuitous situation(s) may occur.

4) I am aware that in invasive medical procedures, such as the one mentioned above, general complications can occur, such as bleeding, infection, cardiovascular and breathing problems and others (...)'.

(...)

Nevertheless, despite the several therapies and treatments given to Mr. Alexandre, such as surgical debridement of the infection, removal of the prosthesis, vacuum bandage, and 'extreme amputation at the thigh level' on 02.22.2016 (pages 1587/2199), remained under the care of the ICU team, evolved

with several complications, dying on 03.13.2016, with 'multiple organ failure, septic shock, soft tissue infection and renal failure' (page 13).

Therefore, it is noted that, by the clarifications provided by the expert and by the answers given to the questions, the presence of comorbidities such as diabetes and immunological weakness, associated with the patient's advanced age (83 years old) were factors that would have hampered the surgical evolution and motivated the infectious condition that affected Mr. Alexandre.

However, if the hospital was already fully aware of the patient's medical history, including having undergone a preoperative clinical evaluation (pages 2454), having verified at the time the aforementioned risks mentioned here by the AACD that increase the probability of complications, the doubt whether the surgery should have been performed, as the plaintiffs pointed out.

Furthermore, it is the doctor's obligation to clarify to his patient everything that is related to the disease and the chances of causing an unexpected effect. The diagnosis, prognosis, procedures, benefits, and adverse reactions, among other information relevant to the treatment, must be very well explained.

In this case, the clarifications contained in the 'Informed Consent Form for Surgical Procedure' (page 2417) were insufficient, as it describes complications similar to those that occur in other surgical procedures such as bleeding, infection, cardiovascular and respiratory problems, but the patient was not informed of the possibility of more serious complications, in the specific case of Mr. Alexandre, as he is a bearer of comorbidities, associated with advanced age, and an increased risk for postoperative infection.

For damages, the amount equivalent to BRL 40,000.00 for each of the plaintiffs is quite reasonable to make up for the damage suffered by the plaintiffs and to repress the act, without implying unjust enrichment to the recipient." (Civil Appeal no. 1045020-32.2016.8.26.0100)[5].

Case 5

The plaintiff underwent surgery on the female reproductive system. Diagnosis of adenomyosis and calculous cholecystopathy was performed, after which total abdominal hysterectomy surgery was indicated. The expert report concluded that there was no failure in the surgical technique.

With that, in fact, the hypothesis was that the claims for indemnity for material and moral damage based on an alleged failure to carry out the surgical procedure itself were unfounded.

Appellate Court conviction: BRL 15,000.00 for damages (expectation and punitive).

Transcript of the decision's excerpt

"However, the cause of action was not limited to the alleged defect in the surgical technique used to treat the Plaintiff, but also included an alleged failure in the information provided by the Defendant to the latter. In fact, in the case of a consumer relationship, the professional service provider has the duty to accurately inform the patient's diagnosis, as well as the procedure to be performed for the treatment and related risks (art. 6th, item III, CDC).

Thus, considering the seriousness of the Defendant's medical conduct and the extent of the damage experienced by the Plaintiff, expectation damages set at BRL 15,000.00 by the lower court judge is considered adequate, an amount that observes the principles of proportionality and reasonableness in the hypothesis, the increase intended by the Plaintiff is not justified." (Civil Appeal no. 1002545-82.2017.8.26.0405)[6]

DISCUSSION

In the experience of the authors in expert charges, the truly informed and prior consent given to and obtained from the patient often does not exist.

In the quantitative aspect, there are two possibilities for informing the patient and obtaining their consent – absent or present. In the qualitative aspect, although the document and the consent exist concretely, it must go through evaluation from the point of view of other logical values: generic or complete.

In the five cases evaluated by the authors, there was the production of expert evidence favorable to health professionals. In most cases in health care, expert evidence is used as sole and sufficient to judge the facts and arrive at a verdict, almost always aligned with the expert opinion.

But in these cases, despite the favorable conclusions of the expert evidence, there was no acquittal from the health professionals.

Due to the mismatch between the expert's conclusions and the decisions, we noted a change in the *status quo* regarding the close relationship between the expert's report and the decisions, and an added insecurity to the professionals. That is, if until then favorable expert evidence led almost inexorably to absolution, this is no longer the case.

CONCLUSION

Although the expert evidence has not pointed out flaws in the surgical procedures, there was a civil conviction of the health care professionals. The analysis of the duty to provide clear and complete information was the responsibility of the High Court

Justices at the São Paulo Court of Appeals, who applied their interpretations on the value and the content of what would supposedly be the informed consent term. From this interpretation, they convicted the health care professionals, regardless of the conclusions of the expert reports. That is, the expert evidence was not an absolute/unique factor in the decisions of the appellate court, since in the five cases, the expert evidence pointed to the correctness of the procedures, but this did not imply an automatic acquittal of the health care professionals.

The judges of the civil courts of the São Paulo Court of Appeals ruled in accordance with the judgment of the Superior Court of Appeals, in which the foreign term blanket consent was first used; although it was not strictly noted in the decisions, its concept was certainly adopted.

A new stage in the litigations in health can be clearly seen, with the appreciation of the patient's autonomy and the duty to inform that is incumbent on professionals, no longer prevailing any conduct that, although correct from a technical point of view, fails to provide tangibility to such principles.

AUTHORS' CONTRIBUTIONS

FRC, AEAR, MLG: All authors have contributed equally to this paper. Their contributions include: Conceptualization, Data curation, Formal Analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, and Writing – review & editing.

NOTES

1. STJ. REsp 1.540.580-5/DF.
2. TJSP. Civil Appeal No. 1014524-26.2019.8.26.0161.
3. TJSP. Civil Appeal No. 1017092-69.2017.8.26.0004.
4. TJSP. Civil Appeal No. 0002255-87.2015.8.26.0653.
5. TJSP. Civil Appeal No. 1045020-32.2016.8.26.0100.
6. TJSP. Civil Appeal No. 1002545-82.2017.8.26.0405.

ERRATUM

https://doi.org/10.1590/1806-9282.20210150ERRATUM

In the manuscript "Chest computed tomography findings of 1271 patients with COVID-19 pneumonia and classifications with different age groups: a descriptive study from Istanbul, Turkey", DOI: 10.1590/1806-9282.20210150, published in the Rev Assoc Med Bras. 2021;67(11):1531-1537:

Page 1533, figure 1

Where it reads:

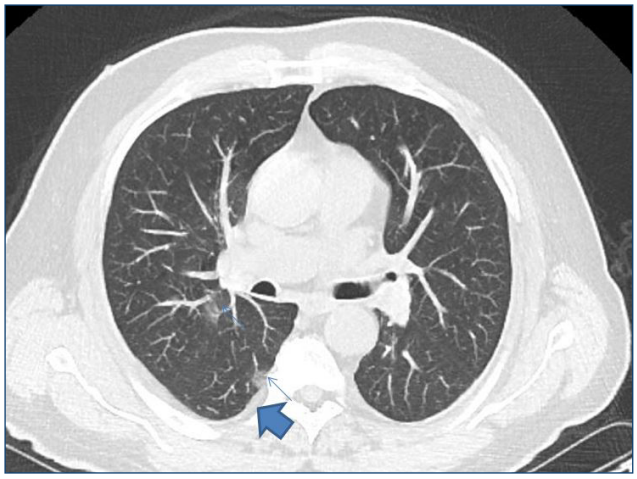
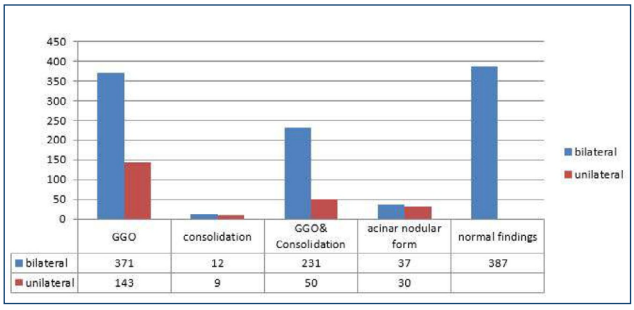


Figure 1. Graph showing the chest computed tomography findings of all patients at the time of presentation to the hospital. The most common chest computed tomography findings were normal. The presence of ground-glass opacity was the second most common lesion group, especially if it was bilateral. The cases with ground-glass opacity and consolidation were the third most common finding. The presence of consolidation alone was a very rare finding that the vast majority were due to atelectasis.

It should read:



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Page 1535, figure 2

Where it reads:

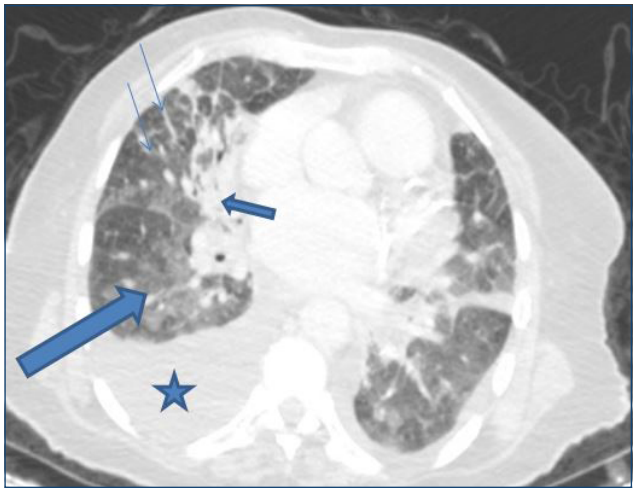
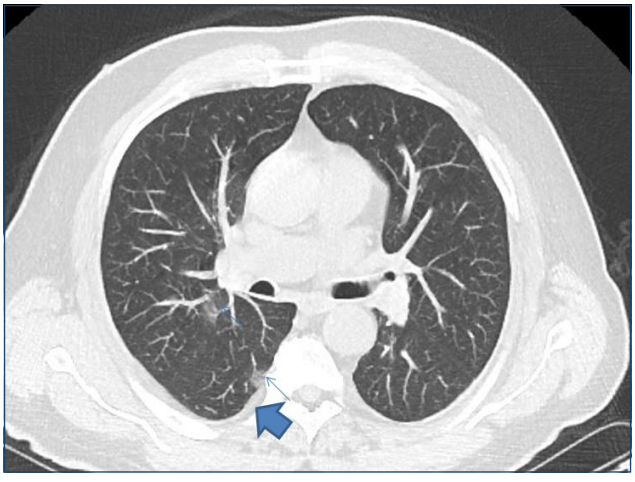


Figure 2. A 52-year-old male has been suffering from weakness and cough for about five days. There are ground-glass opacity adjacent to the fissure and subpleural area (thin blue arrows). Focal pleural thickening is noticed in the right lower lobe adjacent to the lesion (thick blue arrow).

It should read:



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Page 1535, figure 3

Where it reads:

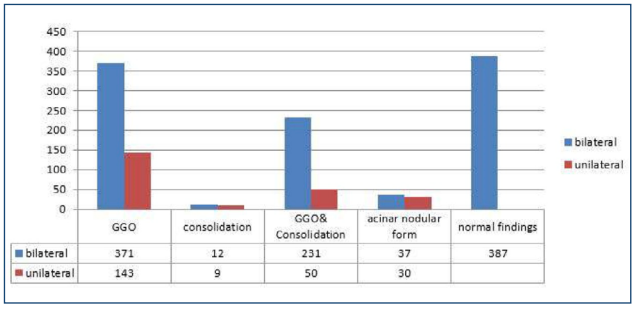
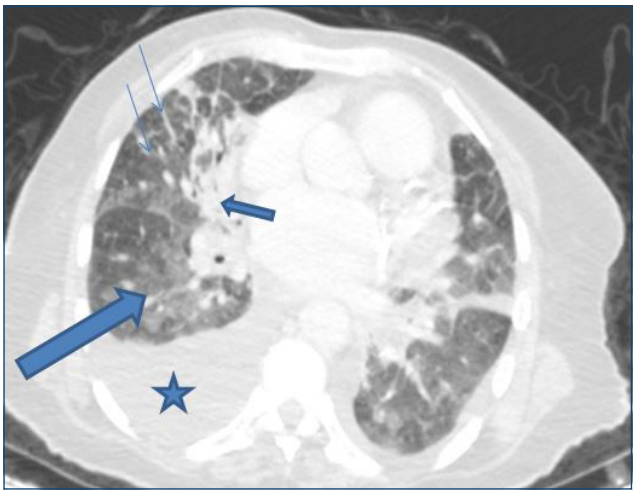


Figure 3. GGO: ground-glass opacity. A 76-year-old female patient has wheezing for the past 15 days and severe abdominal pain for one week. There are bilateral pleural effusion and cardiomegaly (blue stars). Bilaterally moderate interlobular septal thickening is observed (thin blue arrows). There is a focal consolidation in the right middle lobe (small thick blue arrow). Perihilar ground-glass opacity are present (long bold blue arrow). Chest computed tomography findings were classified as atypical findings for COVID-19. Pulmonary congestion findings in chest computed tomography suppress typical radiological findings for COVID-19.

It should read:



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https://doi.org/10.1590/1806-9282.20210476ERRATUM

In the manuscript "Lung ultrasound: an opportunity to increase the accuracy of the physical examination by the nephrologist", DOI: 10.1590/1806-9282.20210476, published in the Rev Assoc Med Bras. 2021;67(11):1729-1734, on page 1731, figure 4:

Where it reads:

Figure 4. Lung ultrasound from a patient with COVID-19 showing an irregular and fragmented pleural line from which a B-line originates.

It should read:

Figure 4. Lung ultrasound from a patient with COVID-19 showing an irregular and fragmented pleural line from which a B-line originates. (Image courtesy of Professor José M. Pazeli Jr.)

ERRATUM

https://doi.org/10.1590/1806-9282.20210940ERRATUM

In the manuscript "Review of the current literature regarding cardiac adverse events following COVID-19 vaccination", DOI: 10.1590/1806-9282.20210940, published in the Rev Assoc Med Bras. 2021;67(12):1751-1757, on page 1751:

Where it reads:
Mert İlker Hayıroçlu

It should read:
Mert İlker Hayıroğlu