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POINT OF VIEW
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# Blurred lines for management of thyroid nodules in the era of atypia of undetermined significance/ follicular lesion of undetermined significance: novel subdivisions of categories IIIA and IIIB in a possible forthcoming The Bethesda System for Reporting Thyroid Cytopathology, 3rd edition; amending versus unnecessary?

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

The management of indeterminate cytology[1-5] of the thyroid nodules, particularly the atypia of undetermined significance or follicular lesion of undetermined significance (AUS/FLUS), has still been one of the most challenging issues in endocrine pathology, neck endocrine surgery, endocrine surgery, endocrinology, and thyroidology. Of note, the estimated risk of malignancy (ROM) for AUS/FLUS was 5–15% in the 1st edition (ed.) of The Bethesda System for Reporting Thyroid Cytopathology (TBSRTC), whereas it was increased to 10–30% in the 2nd ed. TBSRTC. The 2015 American Thyroid Association (ATA) management guidelines for adult patients with thyroid nodules and differentiated thyroid cancer recommend repeat fine-needle aspiration (FNA), molecular testing, surveillance, or diagnostic lobectomy for nodules with AUS/FLUS cytology, after consideration of worrisome clinical and sonographic features[3,4]. The 2015 ATA management guidelines also recommend informed patient preference and feasibility in clinical decision-making ([A17] AUS/FLUS cytology, Recommendation 15A, Weak recommendation-Moderate quality evidence)[3]. Deftereos and colleagues[4] recently reported a worthy study, entitled "Differential outcomes of patients with thyroid FNA diagnoses of AUS/FLUS with and without nuclear atypia: The potential need for separation in the Bethesda System." They proclaimed that the presence or absence of the nuclear atypia is pertinent to the different malignancy rates and, therefore, propounded Category III, TBSRTC might be being divided into two subcategories with different implied ROMs by virtue of the presence of nuclear atypia (consisting of intranuclear pseudoinclusions, nuclear grooving, irregular nuclear contours, and nuclear overlapping). The 2014 Italian Consensus for the Classification and Reporting of Thyroid Cytology (ICCRTC) divided diagnostic category TIR3, indeterminate cytology, into two subcategories, namely, TIR3A (low-risk indeterminate lesion) and TIR3B (high-risk indeterminate lesion), with different ROMs and diverse clinical behaviors. TIR3A is characterized by augmented cellularity with numerous microfollicular structures in a background of poor colloid or scarce cellular structure including predominantly microfollicular groups, also with oxyphilic features, Hurthle cells, harboring estimated ROM of <10%, whereas TIR3B is proclaimed cases with "mild/focal nuclear atypia" at expected higher ROM of 15–30%. Even though

(i) Thy 3A, neoplasm possible, atypia/nondiagnostic, the Royal College of Pathologists, United Kingdom (RCPath, UK);
(ii) TIR3A, low-risk indeterminate lesion, ICCRTC;
(iii) indeterminate or follicular lesion of undetermined significance, Thyroid Cytology Structured Reporting Protocol, 2014 Royal College of Pathologists of Australia (RCPA, 2014); and

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(iv) AUS/FLUS, TBSRTC, 2nd ed. have been compared and matched as if being in the equivalent status, the ROMs are not completely the same for determining the mentioned groups of indeterminate cytology in terms of AUS/FLUS.

For instance, they are <10% for TIR3A, ICCRTC and 10–30% for AUS/FLUS, the 2nd ed. TBSRTC[5]. Of note, actually, the ROM value of TIR3B (15–30%) is compatible with AUS/FLUS (10–30%) rather than TIR3A. As already known, TIR3B involves some additional features consistent with papillary thyroid carcinoma, such as nuclear inclusion, nuclear groove, and overlapping nucleus. To this end, the suggested actions also differ for both TIR3A and TIR3B, i.e., repeat FNA/clinical follow-up for indeterminate cytology whereas surgery for indeterminate cytology with nuclear alterations. Finally, the recommended usual management for AUS/FLUS is repeat FNA, molecular testing, or lobectomy in the 2nd ed. TBSRTC, compatible with both TIR3A and TIR3B, in spite of propounding ROM of Category III, TBSRTC, 2nd ed., is consonant with TIR3B, ICCRTC[5,6]. We recently emphasized whether it is essential to maintain Category III, TBSRTC as a unique and indivisible category, *per se*, among indeterminate cytology of thyroid nodules or not, published in Volume 67, Revista da Associação Médica Brasileira[7]. We currently have mentioned Category III from another perspective and recommended the requirement of zooming in thyroid nodules in suspense, 10–15 mm with repeat cytology, Category III, TBSRTC in Volume 67, Revista da Associação Médica Brasileira[7].

In conclusion, it is critical for the endocrine surgeons and thyroidologists, who stay informed of the growing spectrum of clinical presentation for Category III of TBSRTC, AUS/FLUS cytology, to ensure appropriate clinical care and use of FNA cytology in order to minimize overlooking thyroid malignancy. We postulated that the so-called subdivision concept in Category III, TBSRTC:

(i) Category IIIA: AUS/FLUS without nuclear atypia (AUS/FLUS wo NA) and
(ii) Category IIIB: AUS/FLUS with nuclear atypia (AUS/FLUS w NA)

Within the possible forthcoming Category III, TBSRTC, 3rd ed., the 202X TBSRTC, with different newly established ROMs for each diagnostic category might selectively enrich the different management proposals in thyroidology. As a matter of fact, this issue merits further investigation.

## ACKNOWLEDGMENT

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## AUTHORS' CONTRIBUTIONS

**IS:** Conceptualization, Data curation, Formal Analysis, Writing – original draft, Writing – review & editing. **DS:** Conceptualization, Data curation, Formal Analysis, Writing – original draft, Writing – review & editing.

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SHORT COMMUNICATION
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# "New Homeopathic Medicines" proposal: a database made available in three free-access bilingual digital books

Marcus Zulian Teixeira[1]*

## INTRODUCTION

Homeopathy, a Brazilian medical specialty since 1980, is based on four assumptions, with several lines of research attesting its scientific validity[1]:

(1) principle of therapeutic similitude,
(2) testing of medicines on healthy individuals (homeopathic pathogenetic trials),
(3) prescription of individualized medicines, and
(4) the use of serially diluted and agitated medicines (ultra-diluted and potentized doses). Although much relevance is attributed to ultra-diluted doses, the first two assumptions represent the proper foundation of the homeopathic epistemological model.

In the development of the homeopathic approach to treatment, Samuel Hahnemann (1755–1843) had resource to the phenomenological method of qualitative research to describe the effects of contemporary drugs on the human physiology and ground the therapeutic similitude principle. Hahnemann noted that medicines cause signs and symptoms in healthy individuals similar to the ones exhibited by patients cured with the same medicines. He surveyed the literature and found hundreds of clinical reports by doctors from all times and places, involving many different categories of drugs, which confirmed his finding.

With these evidences and through the application of Aristotelian inductive reasoning (*modus ponens*), Hahnemann outlined the homeopathic healing principle: "for any medicine to cure symptoms in the sick, it must induce similar symptoms in the healthy." By developing a physiological explanation for such "natural healing law," he grounded the therapeutic similitude principle on the "primary action of drugs" and the consequent and opposite "secondary action or vital reaction of the body":

"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,* §63)[2].

Exemplifying this phenomenon, Hahnemann described the primary actions of drugs and the consequent secondary reaction of the body in several physiological systems (Table 1), characterized by the effects opposite to the primary physiological changes (*Organon of medicine,* §59, 65)[2]. The latter leads the body back to the state previous to intervention ("life-preserving power," i.e., modern homeostasis).

Pointing to the unpleasant results of indiscriminate use of medicines with contrary action to the symptoms of disease (*Organon of medicine,* §59-61)[2], Hahnemann called the attention to the fact that the secondary action (vital reaction) of the body might cause undesirable effects ("a relapse – indeed, a palpable aggravation of the malady"), validating homeopathic treatment (principle of similitude) through resource to Aristotelian deductive reasoning (*modus tollens* or affirmation through negation, i.e., the null hypothesis of modern biostatistics).

Since the secondary reaction of the body (opposed to the primary action of the drug) could occur with any category of drugs independently from the dose (ponderable or ultra-diluted), Hahnemann raised the similitude principle to the status of "natural phenomenon" (*Organon of medicine,* §58, 61, 110-112)[2].

Through administration to the sick of the very medicines that induce similar symptoms in the healthy on "homeopathic pathogenetic trials" (similar to our phase I clinical trials)[3], the

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Table 1. Hahnemann's examples of primary action of drug and secondary action (vital reaction) of the body.

| Drugs | Primary action of drug | Secondary action of the body |
|---|---|---|
| Coffee | Excessive vivacity; sleepiness, or insomnia | Sluggishness and drowsiness; somnolence |
| Opium | Profound and stupefied sleep | Sleepiness or insomnia |
| Opium | Constipation | Diarrhea |
| Opium | Analgesia | Hyperalgesia |
| Purgatives or laxatives | Diarrhea | Constipation |
| Cantharides | Polyuria | Oliguria or anuria |

aim of therapeutic similitude is to trigger a curative homeostatic reaction by making the body to react against its own disorders. It should be noticed that the "secondary or vital reaction" designate the ability of living beings to maintain the internal environment constant ("life-preserving power" or homeostasis) through automatic self-adjustment of the physiological processes, ranging from simple cell mechanisms to complex mental functions.

## SCIENTIFIC BASIS OF THE PRINCIPLE OF SIMILITUDE IN MODERN PHARMACOLOGY

In modern scientific terms, Hahnemann's "primary action" corresponds to the "therapeutic, adverse, and side effects" of conventional drugs. In turn, the homeopathic "secondary action or vital reaction" corresponds to the "rebound effect" or "paradoxical reaction" of the body that follows discontinuation of countless categories of drugs that work in a manner opposed (palliative or antagonistic) to the symptoms of disease.

"Rebound effect" is defined as the production of increased negative symptoms when the primary effect of a drug has passed or the patient no longer responds to the drug; if a drug produces a rebound effect, the condition that was used to treat may come back even stronger when the drug is discontinued or lost its effectiveness. Analogously, "paradoxical reaction" is a response opposed to the foreseen effect of a drug. Briefly, we might understand rebound effect as an automatic and instinctive manifestation of the homeostatic mechanisms aiming at reestablishing the original state, altered by the primary action of drugs, resulting in an opposed effect and contrary to the expected one.

The rebound effect appears following discontinuation or withdrawal of drugs, causing manifestations with stronger intensity and/or more frequent than the ones originally suppressed (which distinguish it from relapse of the original disease following the end of the primary action of drugs). These manifestations appear at variable intervals and also have variable duration. As a feature intrinsic to the phenomenon, one should consider a minimum interval of time to have a sound notion of the true magnitude of the phenomenon; this minimum interval corresponds to the full metabolism of drugs or the absence of therapeutic effect (biological half-life). While discontinuation is a requisite for the rebound effect to manifest – since the primary action continues as long as receptors are bounded to the drug – some studies showed that it might also occur along the course of treatment, in cases of therapeutic failure or development of tolerance, tachyphylaxis, or receptor desensitization. In turn, drug tapering avoids abrupt discontinuation and thus minimizes the occurrence of the rebound effect.

Given the epistemological relevance of therapeutic similitude vis-à-vis the remainder of homeopathic assumptions, since 1998, following the Aristotelian deductive reasoning employed by Samuel Hahnemann to scientifically support the law of similars, we have been bridging the gap between homeopathic and conventional pharmacology through the systematic study of the rebound effect of modern drugs[4-7], scientifically confirming the homeopathic postulate (primary action of the drug followed by secondary and opposite reaction of the body) and the homeopathic healing principle.

Tables 2 and 3 list the examples with various categories of drugs illustrating the universal nature of the rebound effect and the principle of similitude[4-7].

These clinical and experimental pharmacological evidences[4-7] show that the characteristics of the rebound effect are similar to the homeopathic secondary action or reaction (*Organon of medicine,* §59, 64, 69)[2]:

(1) it induces a body reaction opposed to and of greater intensity compared to the primary action of drugs;
(2) it takes place after the end of the primary action of the drug, and as automatic manifestation of the body;
(3) it does not depend on the type of drug, dose, treatment duration, or category of symptoms (disease);
(4) its magnitude is proportional to the primary action of the drug; and
(5) it appears in susceptible individuals only (idiosyncrasy).

Table 2. Primary action (therapeutic effect) of modern drugs followed by secondary and opposite reaction (rebound effect) of the body

| Primary action (therapeutic effect) of modern drugs | Secondary reaction (rebound effect) of the body |
|---|---|
| Antiarrhythmic action (adenosine, amiodarone, beta-blockers, calcium channel blockers, disopyramide, flecainide, lidocaine, mexiletine, moricizine, and procainamide) | Rebound exacerbation of basal arrhythmia after discontinuation or withdrawal of drug |
| Antianginal action (nitrates, beta-blockers, and calcium channel blockers) | Paradoxical increase of frequency and/or intensity of angina pectoris |
| Hypotension action (alfa-2 agonists, beta-blockers, ACE inhibitors, MAO inhibitors, nitrates, sodium nitroprusside, and hydralazine) | Paradoxical arterial hypertension |
| Antithrombotic action (argatroban, bezafibrate, heparin, salicylates, warfarin, and clopidogrel) | Rebound thromboembolism |
| Pleiotropic (vasoprotective) action (statins) | Paradoxical endothelial dysfunction |
| Anxiolytic action (barbiturates, benzodiazepines, and carbamates) | Paradoxical anxiety |
| Sedative-hypnotic action (barbiturates, benzodiazepines, morphine, promethazine, and zopiclone) | Increased rebound of agitation, nervousness, restlessness, and irritability |
| Antidepressant action (tricyclic, MAO inhibitors, and selective serotonin reuptake inhibitors) | Paradoxical increase of depressive symptoms |
| Antipsychotic action (clozapine, phenothiazines, haloperidol, and pimozide) | Rebound exacerbation of psychotic manifestations |

Table 3. Primary action (therapeutic effect) of modern drugs followed by secondary and opposite reaction (rebound effect) of the body.

| Primary action (therapeutic effect) of modern drugs | Secondary reaction (rebound effect) of the body |
|---|---|
| Analgesic action (caffeine, calcium channels blockers, clonidine, ergotamine, methysergide, opiates, and salicylates) | Hyperalgesia paradoxical after discontinuation or withdrawal of drug |
| Anti-inflammatory action (steroids, ibuprofen, indomethacin, paracetamol, and salicylates) | Rebound increase of inflammation |
| Diuretic action (furosemide, torasemide, and triamterene) | Paradoxical retention of sodium and potassium with consequent increase of blood volume and arterial pressure |
| Bronchodilator action (short- and long-acting beta-adrenergic agonists, sodium cromoglycate, ipratropium, and nedocromil) | Rebound bronchoconstriction |
| Antidyspeptic action (antacids, $H_2$ antagonists, misoprostol, sucralfate, and protons pump inhibitors) | Paradoxical increase in the production of hydrochloric acid and gastrin |
| Antiresorptive action (bisphosphonates, denosumab, and odanacatib) | Rebound increase of osteoclastic activity causing paradoxical atypical fractures |
| Immunomodulatory action (glucocorticoids, interferon, recombinant monoclonal antibodies, and tumor necrosis factor inhibitors) | Paradoxical effect on the inflammatory and immune response of drug |

Despite this idiosyncratic nature of the rebound effect – which appears in a small proportion of individuals – scientific evidences point to the occurrence of severe and fatal events as a result of the paradoxical reaction of the body following discontinuation of different categories of drugs[5-7]. This corroborates the magnitude of the phenomenon, the need to be duly known by health care providers, and the benefits of its therapeutic application according to the similitude principle.

## "NEW HOMEOPATHIC MEDICINES" PROPOSAL: USE OF MODERN DRUGS ACCORDING TO THE PRINCIPLE OF SIMILITUDE

The basic assumption underlying the homeopathic healing principle is the use of drugs that cause pathogenetic manifestations (signs, symptoms, and physiological or pathological effect) similar to the disorders to be cured. A similar use might be made of any type of drug (natural or synthetic) and in any dose (ponderable or ultra-diluted), provided the therapeutic similitude principle is observed. Thus, modern drugs might be used according to the homeopathic assumptions, provided they induce primary effects (therapeutic, adverse, or side effects) similar to the full set of characteristic signs and symptoms exhibited by patients.

Since 2003, we advocate the use of the rebound effect of modern drugs with curative intent[8-13]. For this purpose, patients are given drugs in ultra-diluted doses, which caused a similar set of adverse events aiming at stimulating the homeostatic reaction of the body against its own disorders.

To make this idea feasible, a *Homeopathic Materia Medica of Modern Drugs* was prepared, in which all the primary or pathogenetic effects (therapeutic, adverse, and side effects) of 1,250 modern drugs described in *The United States Pharmacopeia Dispensing Information* (USPDI)[14] are organized according to an anatomical-functional distribution following the format of the traditional Homeopathic Materia Medica.

To facilitate the choice of the individualized medicine to be prescribed, according to the full set of similar symptoms, a *Homeopathic Repertory of Modern Drugs* was prepared. Here, pathogenetic effects and the corresponding drugs are organized according to the format of the traditional homeopathic repertories, following the aforementioned anatomical-functional distribution.

The proposal entitled "New Homeopathic Medicines: use of modern drugs according to the principle of similitude"[8-13] was described and systematized in a database composed of three distinct works:

(1) Scientific Basis of the Principle of Similitude in Modern Pharmacology,
(2) Homeopathic Materia Medica of Modern Drugs, and
(3) Homeopathic Repertory of Modern Drugs.

## "NEW HOMEOPATHIC MEDICINES" PROPOSAL: A DATABASE MADE AVAILABLE IN THREE FREE-ACCESS BILINGUAL DIGITAL BOOKS

In 2010, in order to allow everyone access to this proposal and its database, these three digital works, totaling thousands of pages, were freely available on a bilingual website (Portuguese and English) prepared on the Adobe Flash Player platform, enabling that this clinical protocol could be analyzed and used by all homeopaths.

Unfortunately, as of 2021, the Adobe Flash Player platform was blocked without offering an alternative to it, preventing colleagues from continuing to have access to that proposal and its database.

Offering an alternative to maintaining this proposal, we have made available the three mentioned works in the format of free-access digital books (PDF), in Portuguese[15-17] and English[18-20] editions. These two editions of three books were indexed in the Virtual Health Library (PAHO, WHO, and BIREME) and are currently accessible to all interested parties:

### Content of the Portuguese edition[15-17]

- Fundamentação científica do princípio da similitude na farmacologia moderna[15];
- Matéria médica homeopática dos fármacos modernos[16];
- Repertório homeopático dos fármacos modernos[17].

### Content of the English edition[18-20]

- Scientific basis of the principle of similitude in modern pharmacology[18];
- Homeopathic materia medica of modern drugs[19];
- Homeopathic repertory of modern drugs[20].

## CONCLUSIONS

To test this proposal, we recently developed a clinical research protocol for the use of potentized estrogen (17-β estradiol) for the treatment of endometriosis-associated pelvic pain, since estrogen causes endometrial hyperplasia or proliferation as adverse event[21]. Reporting significant improvement *versus* placebo in relation to pain, depression, and quality of life[22], this study suggests the validity of this clinical and scientific proposal.

Nevertheless, for this method to be included in homeopathic standard practice, homeopaths need to unite around this project: physicians should apply it in clinical practice and describe the results (case reports), pharmacists should prepare the corresponding homeopathic potentized medicines, and the researchers should design clinical protocols.

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# COVID-19 pandemic and exercising: a cross-sectional study with 1156 patients with fibromyalgia

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## SUMMARY

**OBJECTIVE:** The aim of this study was to analyze the effects of pandemic in the exercising practice and impact of the disease in patients with Fibromyalgia.

**METHODS:** This is a cross-sectional, Internet-based survey answered by 1156 individuals with Fibromyalgia diagnosis. Questions were on epidemiology, social distancing habits, and exercise practice before and after COVID-19 pandemic, including subtypes of exercises (for resistance, flexibility, balance, and strength). The Fibromyalgia Impact Questionnaire was applied.

**RESULTS:** In the whole sample, 57.7% of individuals practiced exercises before pandemic; during pandemic, only 34.8% practiced and 39.6% left this practice. Among those taking quarantine (n=440), 52.9% used to do exercises prior to pandemic; in the pandemic, 28.1% (reduction of 53.2%). The median Fibromyalgia Impact Questionnaire among those who practiced exercises in the pandemic was 73.6 (61.1–83.2) and that among those who did not was 80.4 (71.9–86.9), with p<0.0001. The Fibromyalgia Impact Questionnaire did not change according to the type of physical exercise (p=0.27).

**CONCLUSION:** A high proportion of patients with Fibromyalgia stopped exercising during COVID-19 pandemic; as a result, the impact of the disease during this period was worse among those not practicing exercises.

KEYWORDS: Fibromyalgia. Pandemics. Exercise. COVID-19.

## INTRODUCTION

In November 2019, an increasing number of acute respiratory distress syndrome (ARDS) was reported in Wuhan, a city in the Chinese province of Hubei, and in January 2020, a new coronavirus was identified as the etiological agent responsible for this alarming crescent cases of ARDS[1]. At the same month, the World Health Organization (WHO) declared the outbreak of the novel coronavirus – at the time called 2019-nCoV – a Public Health Emergency of International Concern[1]. The first case reported in Brazil was on February 26, 2020, and, due to the escalating number of confirmed cases, the Brazilian Health Authorities implanted several measures to prevent virus spreading. Due to the nonavailability of effective treatment and due to the lack of an effective vaccine, the main strategy to contain the infection proliferation was to inhibit the person-to-person contamination by social distancing, social isolation, and quarantine[1].

Social distancing is defined as the separation of a sick person with a contagious disease from noninfected persons to protect this second group. Quarantine is an incredibly old and effective tool to control the spread of a contagious infection[2,3]. Although these terms are distinguished by definition, they are interchangeable in public communication and are used likewise in this article[3].

Several studies have been done concerning the effects of social distancing in the health spectrum; significant impact on psychological well-being, emotional health, and practice of

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physical exercise has been reported[4]. Such factors may have had a special influence on the population with fibromyalgia (FM)[5].

FM is a musculoskeletal syndrome characterized by chronic and diffuse pain of unknown etiology[6]. It affects 2.5% of the population worldwide, mostly women aged 30–55 years[7]. In this disease, pain perception is increased and often associated with chronic headache, fatigue, sleep disturbances, depression, anxiety, and nonspecific urethral syndrome[5,7]. The first approach to FM treatment is patient education. Key elements in the patient's education encompass the reassurance that FM is a real illness, information about general aspects of pain and its neurophysiology, and explanations on the influence of stress and mood disorders on the symptoms, among others. The importance of physical exercises in reducing pain and improving general, psychological, and emotional aspects of the patients with FM should also be emphasized[8,9].

In this study, the objective was to analyze the impact of the SARS-CoV-2 pandemic on patients with FM, practice of exercises, and its consequences in the patient's symptomatology.

## METHODS

This is a cross-sectional study based on an Internet questionnaire and was approved by the local Committee of Ethics in Research under protocol 4.106.704 and date June 23, 2020.

To answer the questionnaire, the participants should tick concordance with a consent term. The questionnaire was intended to patients aged 18 years or older with FM and with a previous diagnosis of FM done by a doctor. It was applied from June 24, 2020, to August 31, 2020, through Google Forms using closed, multiples choices questions and questions with short answers and announced on Facebook, Instagram, and WhatsApp groups.

Data collection included epidemiological and clinical information (age, sex, local of residency, disease duration), questions on exercise practice prior to quarantine (including exercise subtype), questions on persistence of exercise practice during pandemic (and, if positive, on exercise subtypes), and questions on isolation. Patients physical exercises practice was classified into exercises for resistance (walking, swimming, etc.), for strength (bodybuilding, weightlifting, etc.), for flexibility (Pilates, stretching, etc.), and for equilibrium (tai-chi, yoga, etc.). The Fibromyalgia Impact Questionnaire (FIQ) was applied. The FIQ is an instrument that measures functional ability and symptoms severity in patients with FM and that has a validated Brazilian-Portuguese version[10]. It consists of 21 individual questions that are answered in a 0–10-point rating scale, with 10 indicating the worst scenario. This instrument measures how the patient feels in the past seven days. The total score ranges from 0–100.

Data were collected and expressed in percentage in frequency tables. Shapiro–Wilk test was used to analyze data distribution; central tendency was expressed in median and interquartile range (IQR) if data were nonparametric and mean and standard deviation (SD) were parametric. Comparison of two series of nominal data was done by Mann–Whitney U test (FIQ values in those practicing and not practicing physical exercises); comparison of more than two was done by Kruskal–Wallis test (FIQ values according to exercises subtypes). The accepted significance was 5%. The software Graph Pad Prism version 6.01 was used for calculations.

## RESULTS

The questionnaire was answered by 1176 individuals. Of these, 20 patients were excluded because they did not confirm that the FM diagnosis was done by a doctor. Table 1 provides details on the duration of FM and epidemiological data of the patients. It is found that most of the patients were middle-aged females and the disease duration was more than 5 years.

Of the 1156 individuals, approximately 116 (10.3%) stayed isolated in 50% or less of the time and 1040 (89.9%) in more than 50% of the time. Again of these 1040 individuals, 440 (42.3%) had some social isolation and were kept quarantine.

In the studied sample, the FIQ score had a median of 79.1 (IQR 69.1–86.3).

Table 2 lists patients who practiced some type of physical activity prior to and during the pandemic. This table also shows the degree of reduction in the physical activity during pandemics.

Table 3 shows the same comparison, but in the sample that kept quarantine. Both Tables 2 and 3 display

Table 1. Epidemiological data and disease duration in 1156 patients with fibromyalgia.

| Median age (years) | 42 (interquartile range 36–48) |
|---|---|
| Females/males (%) | 1099 (95.06)/57 (4.9) |
| Residency* | |
| Midwest region (%) | 151/1152 (13.1) |
| Northeast region (%) | 200/1152 (17.3) |
| North region (%) | 53/1152 (4.5) |
| Southeast region (%) | 486/1152 (42.1) |
| South region (%) | 262/1152 (22.7) |
| Disease duration (year) | |
| >1 (%) | 179 (15.4) |
| 1–4 (%) | 349 (30) |
| <5 (%) | 628 (54.3) |

*Brazilian regions.

that the biggest reduction occurred in those who practice strength exercises.

In the whole sample, the median FIQ was 79.1 (IQR 69.1–86.3). The comparison of FIQ results in those who did any type of exercises with those who did not is shown in Figure 1.

When the domain "pain" of the FIQ was analyzed separately, the obtained results in the whole sample had a median visual analog scale (VAS) of 9 (IQR 8–10). The median VAS of pain in those not practicing exercise was 9 (IQR 8–10) and that in those practicing exercises was 8 (IQR 7–9), with p<0.0001.

In those who kept exercising during pandemic, the median FIQ according to the exercise subtype is given in Table 4. It is found that the subtype of exercise did not change the FIQ.

## DISCUSSION

Data collected in this study show that the exercise practicing in patients with FM was highly influenced by COVID-19 pandemic, with almost 40% of those who practiced regular exercises leaving this practice; in those who kept quarantine, almost half of them stopped exercising. We also found that the impact of the FM was worse in those who did not practice exercise, although no causal relationship can be inferred of this. We also found no differences in the FIQ according to exercise subtype that was done.

The treatment of patients with FM is usually multidisciplinary and difficult as most of the available options offer a modest effect. The 2017 revised European League Against Rheumatism report stated that the only "strong" therapy recommendation for the treatment of FM was exercise[9].

The obtained data on physical exercise neglecting during pandemic is alarming and deserves attention. Patients with FM are known to have low adherence to exercises under normal

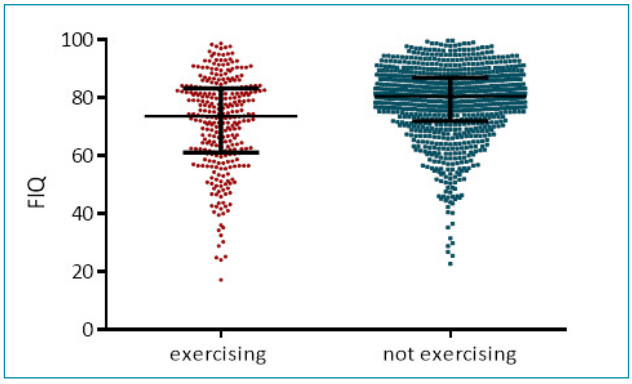


Figure 1. Comparison of Fibromyalgia Impact Questionnaire between individuals exercising and not exercising during COVID-19 pandemic. Exercising: median Fibromyalgia Impact Questionnaire 73.6 (61.1–83.2); not exercising: median Fibromyalgia Impact Questionnaire 80.4 (71.9–86.9) with p<0.0001.

Table 2. Frequency of physical exercising in patients with fibromyalgia prior to and during the COVID-19 pandemic*.

| | Prior to pandemic | During the pandemic | Reduction of exercising during pandemic | Individuals who used to practice but stopped exercising |
|---|---|---|---|---|
| Resistance (%) | 406/1156 (35.1) | 172/1156 (14.8) | 234/1156 (20.2) | 234/406 (57.6) |
| Strength (%) | 155/1156 (13.4) | 43/1156 (3.7) | 112/1156 (9.6) | 112/155 (72.2) |
| Flexibility (%) | 273/1156 (23.6) | 150/1156 (12.9) | 123/1156 (10.6) | 123/273 (30.2) |
| Balance (%) | 44/1156 (3.8) | 30/1156 (2.5) | 14/1156 (1.2) | 14/44 (31.8) |
| Any exercise (%) | 668/1156 (57.7) | 403/1156 (34.8) | 265/1156 (22.9) | 265/668 (39.6) |

*More than one category of physical activity was possible to be checked by the interviewee.

Table 3. Frequency of physical exercising in patients with fibromyalgia prior to and during the COVID-19 pandemic and who kept quarantine (n=440)*.

| | Prior to pandemic | In the pandemic | Reduction of exercising during pandemic | Individuals who used to practice but stopped exercising |
|---|---|---|---|---|
| Resistance (%) | 147/440 (33.4) | 66/440 (15.0) | 81/440 (18.4) | 81/147 (55.1) |
| Strength (%) | 42/440 (9.5) | 9/440 (2.0) | 33/440 (7.55) | 33/42 (78.5) |
| Flexibility (%) | 90/440 (20.4) | 63/440 (14.35) | 27/440 (6.1) | 27/90 (30.0) |
| Equilibrium (%) | 21/440 (4.7) | 11/440 (2.5) | 10/440 (2.2) | 10/21 (47.6) |
| Any exercise (%) | 233/440 (52.9) | 124/440 (28.1) | 109/440 (24.7) | 109/233 (46.7) |

*More than one category of physical activity was possible to be checked by the interviewee.

Table 4. Comparison of Fibromyalgia Impact Questionnaire (FIQ) during COVID-19 pandemic according to exercises subtypes.

| | Median Fibromyalgia Impact Questionnaire (interquartile range) | p-value |
|---|---|---|
| Resistance | 73.4 (57.1–82.4) | 0.27 |
| Flexibility | 71.0 (60.3–83.2) | |
| Strength | 67.1 (50.8–77.6) | |
| Equilibrium | 66.4 (57.5–82.0) | |

circumstances and our results showed that even before pandemic only half of them used to exercise[11]. During pandemic, another half of those who practiced exercise abandoned this routine.

Practicing exercises in public spaces favors social interaction, and this has also beneficial aspect in FM as those patients are prone to depression and anxiety[11]. Therefore, closing of gyms, sports clubs, and public spaces not only affects the physical conditioning but also aggravates the social isolation and its psychological repercussions.

Prior to the pandemic, the most practiced activities were those of resistance, which is a modality of easy access, low cost, safe, and efficient in the treatment of FM[12]. They were found to improve general well-being, symptoms, and cardiovascular capacity better than activities of flexibility[13].

Strength exercises suffered the biggest reduction in practice during pandemic (72.2%), which can be explained by the difficulty of maintaining, for example, weight training – the group's main activity – while the gyms are closed. They consist of a restricted number of series and repetitions movements that utilize a resisted tension and recruit a set of musculotendinous bone structures, with or without load application[13].

Flexibility and balance modalities had the smallest decrease in practice during the pandemic (30.2 and 31.8%, respectively). This is probably due to the fact that most of these activities can be carried on in isolation, outside a specific environment and generally are well-tolerated exercises[14]. Flexibility exercises uses movements with high-level amplitude, using one or more joints with the objective of recruiting the main muscles and joint structures[14]. Balance exercises consist of techniques aiming to acquire stability and body awareness through the practice of postures, respiratory control, and meditative techniques[15].

Regardless of the type of exercise (resistance, strength, flexibility, and balance), the FIQ did not change significantly when one modality was compared to another. The comparison of the different modalities of exercises is difficult mainly due to variations in intensity, frequency, and specific characteristics of each activity and by the fact that patients may combine different subsets of exercises. Therefore, until now, it cannot be said that there is evidence that one exercise is more efficient than another. Despite of this, the FIQ of those who practice any type of exercise was better than those not exercising. It is shown that they do influence pain, sleep, and general well-being, in addition to improving cardiovascular conditioning and overall morbidity and mortality[16,17].

The biological mechanisms involved in the analgesic effects of exercises are not completely understood. They seem to result from an interplay among endogenous opioid system, the endocannabinoid system, and serotoninergic system. This interaction results in a multisegmented decrease in pain sensitivity[16]. Besides, exercises have shown to change brain-derived neurotrophic factor (BDNF) serum levels that are altered in FM and that affects neuronal plasticity acting in the pain sensitization[18].

This study has several limitations. It was based on information that the patients had a FM diagnosis and it was not possible to grade the amount of exercises done. Also, the FM tendency for pain catastrophizing, which is characterized as a negative psychosocial construction of the pain, may suffer influence of other aspects than changes in the physical exercising[19]. Quarantine brought stress, which is a precipitating factor for augmentation in the pain perception, and this may have resulted in a momentary distortion of the FIQ score[19,20]. Finally, stipulating classifications of physical exercises modalities is a great challenge, since each person has particularities for their own practice, and it is not possible to establish a standard approach for the entire sample. However, this work has the merit of showing a great alteration of exercising habits in this sample of patients with FM during pandemic and its possible influence in the impact of this disease.

Educating patients about the importance of physical exercise is fundamental and, at this moment, guiding them to practice physical activity in an adapted way, at home and/or with support via Internet, is essential. It is also extremely necessary that patients who have already adhered to this form of treatment do not fail to pursuit it, mainly in the current atypical circumstances of pandemic and social isolation that, by themselves, tend to worsen the FM symptoms.

## CONCLUSIONS

There was a decreased of physical activity by patients with FM during pandemic, thus worsening the impact of this disease. All subtypes of exercises seem to be beneficial in this context as no differences in the FIQ score could be detected among them.

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## AUTHORS' CONTRIBUTIONS

**IPMM:** Conceptualization, Data curation, Formal analysis, Writing – original draft, Writing – review & editing. **PHKB:** Conceptualization, Data curation, Formal analysis, Writing – original draft, Writing – review & editing. **DBOM:** Data curation, Formal analysis, Writing – original draft, Writing – review & editing. **TLS:** Data curation, Formal analysis, Writing – original draft, Writing – review & editing.

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# Hesitation regarding the COVID-19 vaccine among medical students in Brazil

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## SUMMARY

**OBJECTIVE:** This study aims to know the main determinants of hesitation to the vaccine against COVID-19 mentioned by medical students in Brazil.

**METHODS:** A cross-sectional study with 250 students who answered the online questionnaire between December 18, 2020 and January 8, 2021.

**RESULTS:** Most students (84%) mentioned the intention to take anti-COVID vaccine and 14% were hesitant. Information provided by governments (59.2%), the pharmaceutical industry (54.4%), and the press (51.6%) were the items that most generated vaccine hesitation.

**CONCLUSION:** In the context of the COVID-19, vaccine hesitation is an additional concern because adherence to vaccination is a recurring challenge. The category of contextual influences predominated among the main determinants of anti-COVID vaccine hesitation expressed by medical students in Brazil, disfavoring vaccine adherence in this public.

KEYWORDS: COVID-19. Vaccination refusal. Health literacy. Students, medical.

## INTRODUCTION

The vaccine against COVID-19 is the preventive measure most awaited by the global population. Immunizers are being developed using various technological platforms, such as messenger RNA, and synthetic and modified particles, such as viruses, among others[1], originating immunobiologicals with different mechanisms of action for the same purpose.

The dissonance in this context is that a vaccine takes at least an average of 10 years to be released for consumption[2], which was not the case with anti-COVID vaccines. As a result, the increasing speculation contributed to raising doubts and divided opinions about accepting or refusing the vaccine.

In Brazil, the acceptance of vaccines has been decreasing since 2016, which may be due to experiences with vaccination, low health literacy, perception of the pharmaceutical industry, and lack of information. As a result, vaccine-preventable diseases have increased in different Brazilian regions[3].

Vaccination hesitancy, defined as the delay in accepting or refusing vaccination[4], is an additional concern in the context of COVID-19. This is because vaccination adherence is a recurrent challenge in different segments of society, including Brazilian health professionals, which is verified by the low vaccine coverage against diseases such as hepatitis B[5].

Although health care professionals and students have basic training on vaccines in general, a recent review study with medical students[6] revealed a lack of knowledge about their own vaccine status. Another study[7] with 39 physicians and 53 students from a medical school in Brazil found that most were in favor of nonmandatory vaccination, considering it reasonable

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to respect the desire of someone who refused to have themselves or their children vaccinated.

According to the National Curricular Guidelines for medical programs in Brazil[8], students must experience health services from the early semesters, thus being exposed to a higher risk of immunopreventable diseases. Intensified by the COVID-19 pandemic, this problem raised the following question: how do medical students formulate their opinions regarding anti-COVID vaccination? The importance of exploring this topic lies in the fact that their vaccination aims at both protection against contamination during the program's practical activities and their inclusion in the taskforce of health professionals working during the pandemic.

Moreover, even knowing that vaccine hesitancy varies according to the time and is specific to each context and that vaccine literacy resulting from necessary information ensures the right and autonomy in decision-making[9], this study identified the main determinants of hesitancy regarding the COVID-19 vaccine reported by medical students in Brazil.

## METHOD

This cross-sectional study uses the opinion survey method without identification of participants, as provided for in Resolution 510/2016 of the National Health Council, which was conducted by posting an electronic questionnaire and a request for disclosure on a fanpage of directories of medical students. Virtual snowball sampling, which uses social media for data collection, was employed.

Data collection began on December 18, 2020, after the Supreme Court ruled that COVID-19 vaccination would be compulsory in nature in Brazil, and ended on January 8, 2021, when the Butantan Institute and Fiocruz submitted an authorization request to Anvisa for the emergency use of the CoronaVac/Sinovac and Oxford/AstraZeneca vaccines in Brazil.

The data obtained from the questionnaires were analyzed using BioEstat 5.0 software. Descriptive statistics, Pearson's chi-square, and G-tests (Wiliams) were used to evaluate the associations between the variables, and the results were classified according to the three categories of influences proposed by the World Health Organization (WHO) Matrix of Determinants of Vaccine Hesitancy:

(1) contextual, encompassing aspects of communication mediated by influential people, policy, and perception of the pharmaceutical industry;
(2) individual and group, through personal or family experiences with vaccines; and
(3) vaccine/vaccination-specific, encompassing topics related to immunizers[4].

## RESULTS

The study included 250 students, of which 58.5% (n=147) were females, aged between 18 and 25 years (n=209; 83.6%), belonging to the basic cycle (n=125; 50.0%), clinical cycle (n=102; 40.8%), and internship (n=23; 9.2%) and attending public higher education institutions (HEIs) (n=146; 58.4%). The Northeast region had the highest percentage of participants (n=125; 50%), followed by the Southeast (n=66; 26.4%), South (n=17; 6.8%), North (n=14; 5.6%), and Midwest (n=08; 3.2%). Cases of COVID-19 or infected family members were reported by 66.4% of students, and 35.2% said they knew someone who had died from the disease.

Most students (84%) expressed the desire to be vaccinated, and 14% were hesitant. Information provided by governments (59.2%), the pharmaceutical industry (54.4%), and the press (51.6%) were the items that caused most vaccine hesitancy. Conversely, 93.2% of the participants showed confidence in the development process of anti-COVID vaccines, and 66.4% were in favor of mandatory vaccination (Figure 1).

Students from public and private HEIs reported doubts about the new coronavirus (61 and 64.4%; p=0.0004) but stated that they would have the vaccine when it became available to the population (89 and 76.9%; p=0.04) (Table 1).

## DISCUSSION

The main determinants of hesitancy regarding the COVID-19 vaccine revealed the great variety of aspects that influence how the medical students viewed vaccination.

Similar to other studies[10], the high percentage of students in favor of vaccination indicates confidence in immunobiologicals and the perception of risk of acquiring the disease when not immunized. Conversely, differing from other studies[11], the hesitant students cited topics peculiar to the pandemic scenario in Brazil, highlighting political issues involving access to vaccines, which demonstrates that contextual influences overlay individual and specific experiences of vaccination.

Although these results are encouraging when compared to those in a medical school in the United States, where 23% were undecided[12], 2% of students who were against vaccination should be considered. This may be due to the anti-vaccination movement, which, although having greater influence in North America and Europe, may constitute a potential barrier to the effectiveness of vaccination in Brazil[13]. When attending health services in training activities, these students are more vulnerable to contagion when they are not vaccinated and are potential hidden reservoirs for COVID-19[14].

Contextual influences were expressed through the low level of confidence in some information media in Brazil, with

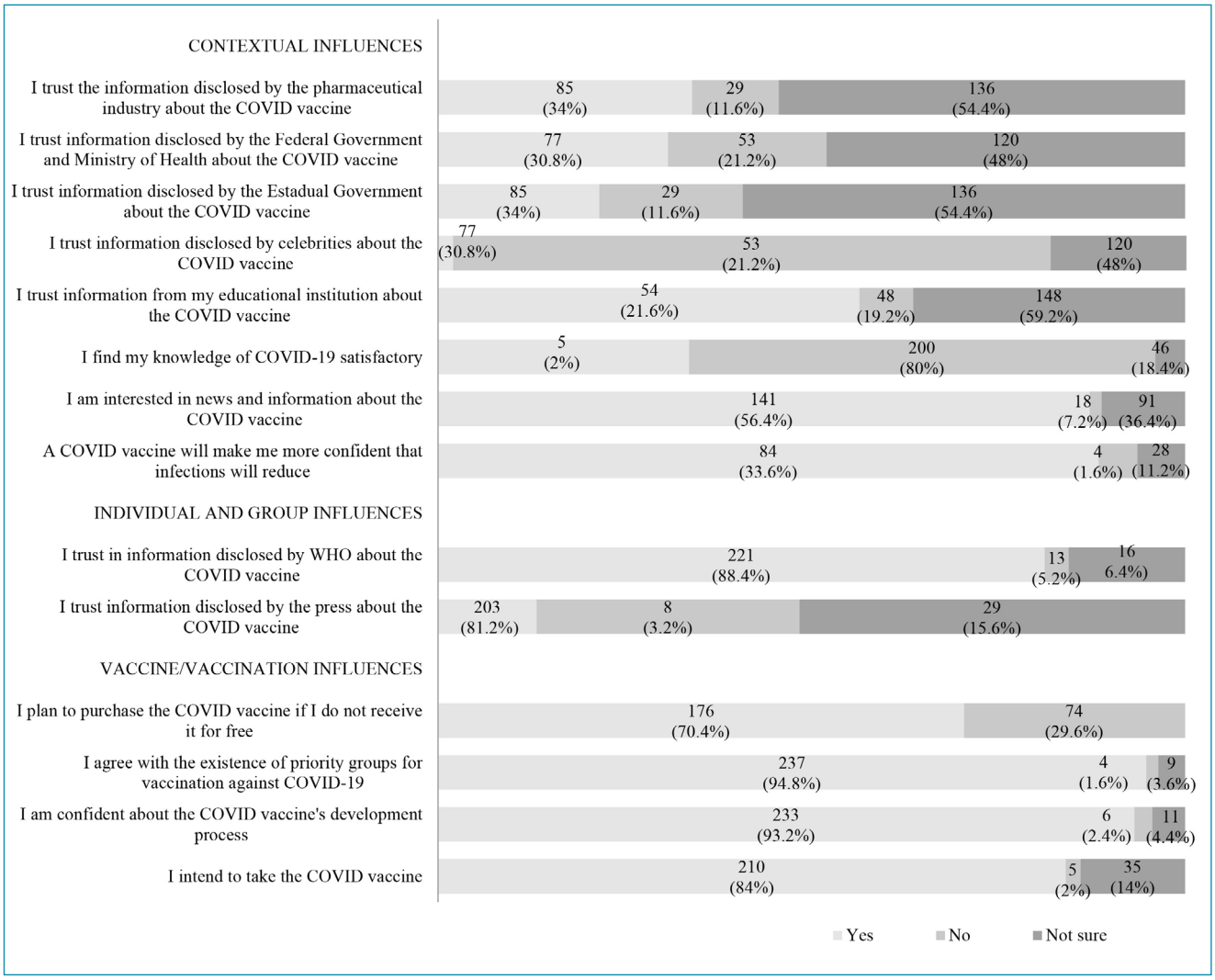


Figure 1. Vaccine acceptance, refusal, and hesitancy among medical students.

hesitancy being mostly attributed to the pharmaceutical industry and the three spheres of government. Similar to the results revealed in a study with Americans, this aspect, which considered the support from the government of the country as a way to increase the acceptance of vaccination[15], surpasses the technical and individual limits of the vaccine decision processes and establishes deep interfaces with economic and political issues.

Students considered HEIs reliable sources of information about COVID-19. These findings support the social responsibility agenda of these institutions, which are strategic locations for the promotion of educational interventions that increase behavioral trust in this group[16], as adolescents and young adults are included as an eligible group for vaccination in some Brazilian states.

Knowledge about COVID-19 and interest in the topic also exerted a contextual influence on the students. These results are similar to a study conducted in France with students from 32 medical universities. More than one-third were not confident in explaining the risks and benefits of vaccines to patients, revealing gaps in medical education[17].

Information provided by the WHO and news reported by press media also affected the students' vaccination decisions. This lack of synchrony between information and viral contamination leads to government instability and misinformation, accelerating the epidemic outbreak and weakening trust in institutions[18]. Such situations, exacerbated by fake news that raise doubt on the scientific validation of vaccines, can make students less prone to follow legitimate health guidelines and take proven preventive measures[19].

The students' personal reports about the existence of cases and deaths of family members from COVID-19, which was also found in other studies[20], may have influenced their opinions,

Table 1. Association between items regarding the COVID-19 vaccine and the groups of interest.

| Item | Groups | Answers | | | |
|---|---|---|---|---|---|
| Do you consider your knowledge about COVID-19 satisfactory? | Type of program | Yes (%) | Not sure (%) | No (%) | p-value* |
| | Public | 47 (32.2) | 89 (61.0) | 10 (6.9) | 0.0004 |
| | Private | 37 (35.6) | 67 (64.4) | 0 | |
| | Total | 84 (33.6) | 156 (62.4) | 10 (6.9) | |
| Would you have a vaccine that has been authorized for use in Brazil? | Type of program | Yes (%) | Not sure (%) | No (%) | p-value* |
| | Public | 130 (89) | 14 (9.6) | 02 (1.4) | 0.04 |
| | Private | 80 (76.9) | 21 (20.1) | 03 (2.9) | |
| | Total | 210 (84.0) | 35 (14.0) | 05 (2.0) | |
| | Period of the program | Yes (%) | Not sure (%) | No (%) | p-value* |
| | Basic | 103 (82.4) | 20 (16.0) | 02 (1.6) | 0.68 |
| | Clinical | 86 (84.3) | 13 (12.8) | 03 (2.9) | |
| | Internship | 21 (91.3) | 02 (8.7) | 0 | |
| | Total | 210 (84.0) | 35 (14.0) | 05 (2.0) | |
| Do you trust the information given by our educational institution about COVID vaccines? | Period of the program | Yes (%) | Not sure (%) | No (%) | p-value* |
| | Basic | 73 (58.4) | 46 (36.8) | 06 (4.8) | 0.47 |
| | Clinical | 56 (54.9) | 35 (34.3) | 11 (10.8) | |
| | Internship | 12 (52.2) | 10 (43.5) | 01 (4.5) | |
| | Total | 141 (56.4) | 91 (36.4) | 18 (7.2) | |
| Do you consider that vaccination against COVID-19 should be mandatory? | Period of the program | Yes (%) | Not sure (%) | No (%) | p-value* |
| | Basic | 85 (68.0) | 20 (16) | 20 (16) | 0.50 |
| | Clinical | 67 (65.7) | 13 (12.8) | 22(21.6) | |
| | Internship | 14 (60.9) | 06 (26.1) | 03 (13) | |
| | Total | 166 (66.4) | 39 (15.6) | 45 (18.0) | |

*G test (Williams).

favoring vaccine acceptance, as the still persistent epidemiological panorama of COVID-19 indicates that without specific vaccine protection, the disease can quickly spread among people and cause irreparable damage to society[21].

Specific aspects of the vaccine/vaccination were the factors that contributed less to hesitancy. The development of vaccines was a positive aspect for vaccine acceptance, even with some of the students disbelieving or being unsure about the manufacturing process of immunizing agents. These results differ from the hesitations that would be expected in this group, such as those reported by health professionals who mentioned side effects, vaccine efficacy, and virus mutation potential as the main determinants[22,23].

None of the students indicated the acquisition of an anti-COVID vaccine with their own financial resources, if it was not provided by the public administrators, as a cause for hesitancy, but this item was rejected by some students. Situations that make it difficult to offer these vaccines include the absence of national planning, slow negotiations with manufacturers, and political disputes between the federal government and state managers. Added to the presidential discourse that presents vaccination as an individual choice and encourages the population to distrust a particular immunizing agent[24], these obstacles place the pandemic in a challenging context that impact their willingness to take the vaccine, even if it is necessary to acquire it with their own financial resources.

Although most of the students agreed with the prioritization of groups for vaccination against COVID-19, some were hesitant. It is clear that a vaccination plan with priority groups was established due to the insufficient number of immunizations purchased by the federal government. However, even though it is acceptable the most vulnerable are vaccinated first, efforts

should be made to ensure a vaccination scheme that encompasses all the population, given that, even among health professionals, coverage with the second dose is still insufficient, as it represents 55.9% of those who received the first dose[25], indicating poor vaccination performance in Brazil.

Even in the face of this increasingly worrying scenario, the existence of an anti-COVID vaccine by itself will not be enough to eliminate vaccine hesitancy among populations[24], as the elements involved in this process bring the confrontation between technical-scientific, sociocultural, political, and economic aspects to the core of the pandemic, resulting in the temporal unpredictability that will mark its end.

## CONCLUSIONS

This study revealed multifaceted aspects that influence the opinions of medical students on the reliability of anti-COVID vaccines and acceptance of vaccination. Contextual influences were the main determinants of expressed hesitancy, to the detriment of vaccine adherence in this group. These findings indicate the need for strategies that promote vaccine literacy and vaccination, contributing to students' biosafety in teaching and learning scenarios.

## AUTHORS' CONTRIBUTIONS

**ÍESC:** Conceptualization, Data curation, Formal analysis, Methodology, Writing – original draft, Writing – review & editing. **PRPB:** Conceptualization, Data curation, Formal analysis, Methodology, Writing – original draft, Writing – review & editing. **MRCM:** Conceptualization, Data curation, Formal analysis, Methodology, Writing – original draft, Writing – review & editing. **JGBAR:** Formal analysis, Writing – original draft, Writing – review & editing. **ELC:** Formal analysis, Writing – original draft, Writing – review & editing. **MSC:** Formal analysis, Writing – original draft, Writing – review & editing.

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ORIGINAL ARTICLE
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# Immunogenicity after CoronaVac vaccination

Eda Çelik Güzel[1]*, Aliye Çelikkol[2], Berna Erdal[3], Nuriye Sedef[1]

## SUMMARY

**OBJECTIVE:** This study aimed to investigate the seropositivity of CoronaVac-SinoVac vaccination in severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) risk factors and comorbidities.

**METHODS:** Immunoglobulin (IgG) antibody responses were examined on the 21st day after the second dose of CoronaVac-SinoVac 6 µg vaccine on the 28th day. SARS-CoV-2 IgG antibody levels were measured by using the enzyme-linked immunosorbent assay method in vaccinated health care workers (n=134) (Group I), vaccinated polymerase chain reaction (PCR) (+) who had coronavirus-19 (COVID-19) disease (n=21) (Group II), and unvaccinated PCR (+) (n=28) (Group III) participants. Subgroups were formed in Group I according to the presence of COVID-19 risk factors and comorbidities (diabetes mellitus, cardiovascular disease, and asthma/allergy) and demographic data.

**RESULTS:** Seropositivity rates were 95.5, 100, and 89.3% for Groups I, II, and III, respectively. IgG antibody levels were found significantly higher in the group between the ages of 20–30 in group I compared to those aged 31–50 and over 50 (both $p<0.01$). It was found significantly higher in normal-weight individuals than in the overweight and obese group (both $p<0.01$). IgG antibody levels were found significantly lower in people with cardiovascular disease and diabetes mellitus compared with those who did not ($p<0.05$ and $p<0.001$, respectively). There was a negative correlation between IgG antibody response values and body mass index and age in Group I ($r= -0.336$, $p<0.001$ and $r= -0.307$, $p<0.001$, respectively).

**CONCLUSION:** IgG antibody values decrease with age and with increasing body mass index. The presence of comorbidities (i.e., diabetes mellitus and cardiovascular disease) decreased COVID-19 IgG antibody values.

KEYWORDS: COVID-19 virus disease. Risk factors. Comorbidity. Immunogenicity, vaccine.

## INTRODUCTION

Coronavirus-19 disease (COVID-19) pandemic caused by severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) has infected more than 110 million people and killed approximately 2.5 million people as of March 2021[1].

Post-vaccine antibody tests against SARS-CoV-2 showed that adequate levels of neutralizing anti-SARS-CoV-2 S1-specific immunoglobulin (anti-S-IgG) antibodies were formed[2-5]. One of these vaccines is the CoronaVac-SinoVac (inactivated SARS-CoV-2) (Sinovac Life Sciences, Beijing, China) vaccine, whose phase 3 trials have been completed. Phase 1 clinical trials were conducted as dose escalation, and the highest antibody response occurred after two doses of 6 µg vaccine (0 and 28 days). In the same study, in which phase 2 clinical trials were conducted to increase vaccine production capacity, the seroconversion of neutralizing antibodies was 97% in the 3 µg group and 100% in the 6 µg group. However, the titers of neutralizing S IgG produced after vaccination were lower than the

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serum S IgG levels of those who had the disease[4]. This is a disadvantage of inactivated vaccines and may affect the response to vaccination of patients in the risk groups for COVID-19.

The aim of this study was to monitor the anti-spike IgG antibody response after CoronaVac vaccination against COVID-19 disease and to investigate the antibody response based on risk factors associated with COVID-19 disease and comorbid conditions.

# METHODS

## Study design and participants

This prospective study was conducted on approval of the Ethics Committee (Protocol number; 2021.55.02.18). In this study, we included 183 volunteers who applied for the vaccination in University Hospital. CoronaVac-SinoVac vaccine was administered intramuscularly in two doses of 6 µg (0 and 28 days). People who applied for the vaccination in the outpatient clinic for the second dose 28 days following the first dose and whose blood sample was obtained 21 days after the second dose (n=134) constituted Group I. PCR (+) patients who have had the disease and also had the first and second dose of the vaccine constituted Group II (n=21). PCR (+) patients who have had COVID-19 disease but are unvaccinated (n=28) constituted Group III. To examine the IgG antibody response, 2 mL of whole blood samples were taken 21 days after the second dose of the vaccine from Groups I and II, and immediately from Group III. After centrifugation at 3,000 rpm for 10 min without delay, the samples were stored at -80°C until further analysis. A COVID-19 positive group (Groups II and IIII) was formed of patients whose quantitative reverse transcription PCR (RT-qPCR) results were positive. Sample collection time was within two months on average from a positive PCR result. Group I was divided into three subgroups with age as 20–30 years, 31–50 years, and above 50 years old, and with body mass index (BMI) as normal (18.5–24.9), overweight (25–29.9), and obese (>30). Smoking, vitamin supplement, regular exercise, and regular sleep characteristics of group I were obtained through a questionnaire. As shown in Table 1, participants were grouped with chronic disease, asthma, diabetes mellitus (DM), cardiovascular disease (CVD), diagnosis, or/and treatment in the last year obtained from the hospital electronic archive and viral load according to working place in the hospital.

Anti-SARS-CoV-2 human IgG measurement was performed using the enzyme-linked immunosorbent assay (ELISA) kit (DIA. PRO, Sesto San Giovanni, Milan, Italy). Serum samples were incubated in wells pre-coated with recombinant nucleocapsid

Table 1. Demographic characteristics of vaccinated participants, Eda Celik Guzel.

| | Group I (n=134) | Group II (n=28) | Group III (n=21) |
|---|---|---|---|
| Age (years) | 39.04 (25–60) | 34.76 (24–52) | 29.07 (21–43) b***, c* |
| Gender (F/M) | 73/61 | 14/14 | 11/10 |
| BMI (kg/m$^2$) | 26.95±5.6 | 28.21±7.08 | 24.75±3.68 c* |
| Smoking (-/+) | 101/54 | 28/0 | 16/5 |
| Regular sleep (-/+) | 52/82 | 12/16 | 11/10 |
| Regular exercise (-/+) | 111/23 | 20/1 | 1/20 |
| Vitamin supp. (-/+) | 65/69 | 13/8 | 13/8 |
| Comorbidity | | | |
| CVD (-/+) (%) | 91/43 | 27/1 | 2/19 |
| Diabetes mellitus (-/+) | 100/34 | 0/28 | 3/18 |
| Asthma/allergy (-/+) | 94/40 | 21/7 | 2/19 |
| Viral load (high/low) | 67/67 | 28/8 | 17/4 |
| COVID-19 IgG efficacy | | | |
| COVID-19 IgG levels | 7.15 (0.19–12.8) | 9.36 (4.58–2.41) a** | 7.34 (0.01–12.5) c* |
| COVID-19 IgG (-/+) (%) | 6/128 (95.5) | 3/25 (89.3) | 0/21 (100) |

*p<0.05; **p<0.01; ***p<0.001. a: between Groups I and III; b: between Groups I and II; c: between Groups II and III. Group I: two doses vaccinated individuals without COVID-19 disease or contact. Group II: two doses vaccinated individuals have had COVID-19 disease before. Group III: unvaccinated people who have had COVID-19. F: Female; M: male; BMI: body mass index; Vitamin supp.: vitamin supplementation; CVD: cardiovascular diseases.

and spike proteins of SARS-CoV-2. Results were calculated as the ratio between the optical density of the sample and the optical density of the negative control in the kit and expressed as arbitrary units (AU). The sample positivity cut-off value for anti-nucleocapsid and/or anti-spike antibodies was determined by the manufacturer as AU>1.1.

Statistical analysis was performed using SPSS IBM 18.0 (SPSS Inc., Chicago, IL, USA). The distribution of the data was determined using the Kolmogorov–Smirnov test. The independent samples *t*-test was used to compare normally distributed variables, and the Mann–Whitney U test was used to compare non-parametric distributed variables.

## RESULTS

In the investigation of COVID-19 IgG antibody levels, a statistically significant difference was found between Groups I and II and between Groups II and III (p<0.01 and p<0.05, respectively). Antibody formation was highest in Group II. On the 21st day after vaccination, the antibody positivity rate was 95.5% for Group I and 100% for Group II. The antibody positivity rate was 89.3% in Group III, who were not vaccinated (Table 1).

Group I was subgrouped according to age, gender, BMI, smoking, vitamin use, and working in areas with high viral load and comorbid diseases, and the relationship with antibody levels was evaluated. Mean antibody levels were 8.91 (n=29, [1.98–12.80]) in the 20–30 age group, 6.86 (n=85, [0.19–12.78]) in the 31–50 age group, and 5.46 (n=20, [0.68–10.51]) in the >50 age group. Antibody response levels were significantly higher in the 20–30 age group compared with the 31–50 and >50 age groups (p<0.01 for both) (Figure 1A). In BMI subgroups, antibody levels were significantly higher in normal-weight subjects (n=60, [8.36 (1.09–12.80)]) compared with the overweight (n=39, [6.78 (1.31–12.78)]) and obese groups (n=35, [5.47 0.19–11.7]) (p<0.01 for both) (Figure 1B).

Antibody response levels of those in Group I working in areas with high viral load were significantly higher compared with those working in areas with low viral load (n=66, 8.31 [1.40–12.80]; n=67, 6.01 [0.19–12.11]; respectively, (p<0.001) (Figure 2).

Antibody response levels were significantly lower in participants with comorbid diseases in Group I (n=95, 6.2 [0.19–12.26]) compared with those without comorbid diseases (n=39, 9.45 [1.98–12.80]) (p<0.001). Antibody levels were significantly lower in individuals with CVD compared with those without CVD (n=43, 6.16 [1.40–12.80]), (n=91, 7.61 [0.19–12.26]), respectively; p<0.05). People with DM had significantly lower antibody response levels compared with those without DM (n=34, 4.89 [0.19–10.74]; n=100, 7.92 [1.21–12.8], respectively; p<0.001) (Figure 2).

A negative correlation was found between IgG antibody response and BMI and age in Group I (r= -0.336, p<0.001 and r= -0.307, p<0.001, respectively).

## DISCUSSION

Group II had the highest IgG antibody response after vaccination. Antibody levels of the vaccinated PCR (+) group were higher than the PCR (+) group without vaccination (p<0.05). Similar to our findings, Jabal et al.[5] showed that in single-dose BNT162b2 mRNA COVID-19 vaccination, post-vaccination

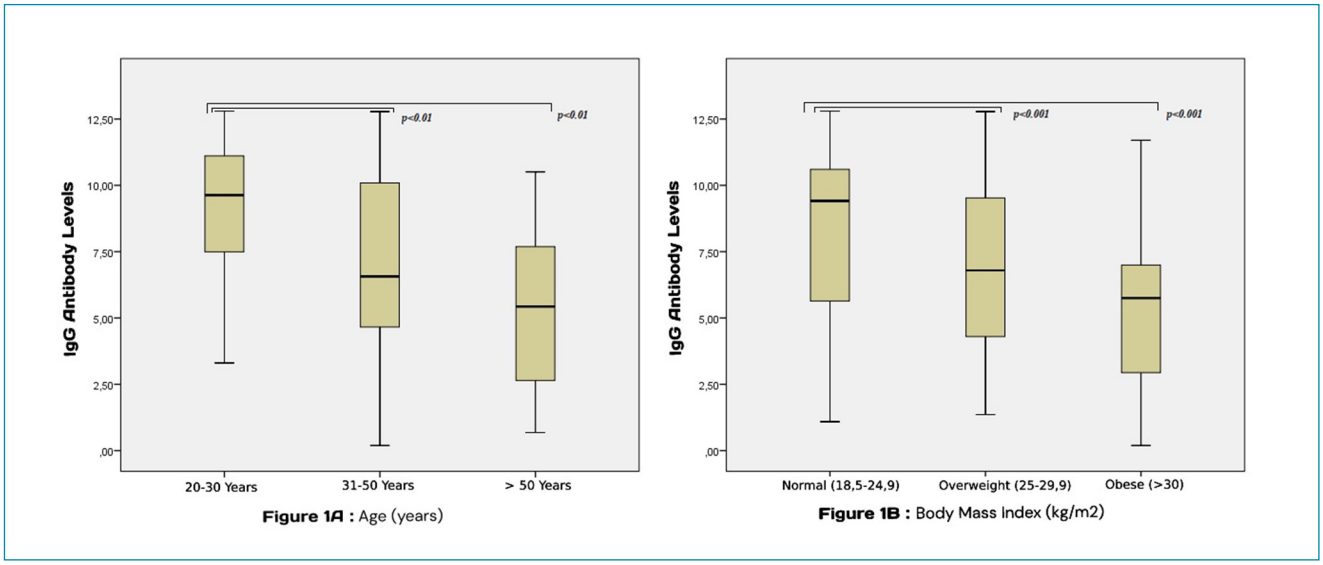


Figure 1. COVID-19 IgG levels in age (A) and body mass index (B) sub-groups of vaccinated Group 1, Eda Celik Guzel.

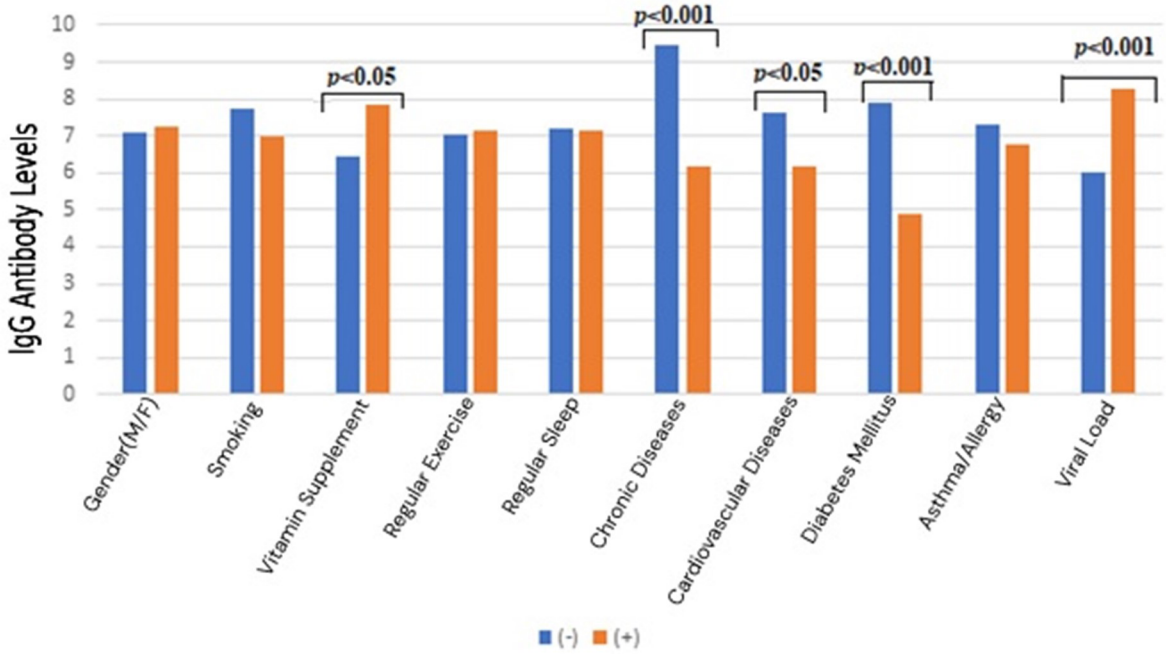


Figure 2. Differences of COVID-19 IgG antibodies Levels in sub-groups of vaccinated Group I, Eda Celik Guzel.

COVID-19 IgG antibody levels were much higher among those with previous evidence of disease. Furthermore, the antibody levels after vaccination in Group I reached values nearly equal to Group III (Table 1). It is a remarkable result that on the 21st day after vaccination, the antibody positivity rate of Group I (95.5%) was higher than that of Group III (89.3%) (Table 1). These results confirm that the effectiveness of the CoronaVac vaccine is very high. At the same time, our findings were consistent with the results in the CoronaVac phase two study[4].

Studies examining the effect of risk factors and comorbidities on antibody titer in vaccinated persons are quite limited. In this study, when Group I was subgrouped according to age, antibody levels were significantly higher in the 20–30 age group compared with that in the 31–50 and over 50 age groups ($p<0.01$ for both) (Figure 1A). Contrary to these results, preliminary results of the CoronaVac phase 1 study reported that antibody responses did not differ between the ages of 18 and 59 years[2]. CoronaVac phase two results also reported that antibody response did not differ in individuals over 60 years old, similar to the phase 1 study. Similar to our results, vaccine studies other than CoronaVac in the literature report that antibody formation decreases with age. Antibody titers were reported to decrease with age after a single dose of BNT162b2 mRNA COVID-19 vaccination of health care workers in Israel ($p<0.001$). Xia S et al.[3] found that the neutralizing antibody response against SARS-CoV-2 after BBIBP-CorV vaccination was lower in the >60 age group compared with the 18–59 age group. In the examination of age, the antibody response was decreased in older age groups, which supports our study results[6,7]. In the evaluation of other vaccination studies, a negative correlation was detected between COVID-19 IgG antibody levels and age as this study supports this finding.

COVID-19 IgG antibody levels of normal-weight individuals in Group I were significantly higher than overweight and obese individuals ($p<0.01$ for both) (Figure 1B). Therefore, it was found that antibody response decreases with increasing BMI. This result can be interpreted as either the insufficiency of the immune mechanisms in obesity or the insufficient antibody response of the vaccine dose in obese individuals. A similar finding has not yet been reported. In the investigation of obesity, Aude Richard et al.[6] reported no significant relationship between BMI and seropositivity. However, they reported that obese women tended to have higher seropositivity compared with normal BMI. The existence of a negative correlation between IgG antibody levels and BMI in this study suggests that this mechanism may be different during the vaccination process.

There is a linear relationship between maintaining a healthy life and immune response[8]. Antibody levels were significantly higher in vitamin C and vitamin D users ($p<0.05$) (Figure 2). During the COVID-19 pandemic, the use of nutrients that strengthen immunity, such as vitamins C and D, zinc, etc., increased. However, studies supporting the effect of dietary

supplements in the prevention of COVID-19 are not clear and convincing[9]. In this study, 51.5% of the volunteers in Group I were taking vitamin supplements.

Another remarkable point is that among the health care professionals in Group I, the antibody levels of those working in areas with high viral load (i.e., emergency clinic, pandemic clinic, and pandemic ward) were significantly higher than those who did not work in these areas ($p<0.001$) (Figure 2). According to this result, it was thought that encountering a low viral load would keep the immune response ready, and a better antibody response can be generated after vaccination. In fact, Jabal et al.[10] found that health care workers with previous evidence of infection had higher post-vaccination IgG levels, which supports this finding. Compared with the antibody response following the recovery of patients with COVID-19, a history of close contact with a person infected with SARS-CoV-2 was found to increase the probability of seropositivity approximately five times[6].

There were no studies on the COVID-19 vaccine in the literature investigating the effects of comorbid diseases on antibody responses. Antibody levels were significantly lower in participants who had comorbid diseases in Group I ($p<0.001$). At the same time, antibody levels were significantly lower in individuals with CVD ($p<0.05$) and DM ($p<0.001$) (Figure 2). However, antibody response following the recovery of patients with COVID-19 was investigated in various comorbid conditions, and it was reported that those with comorbidities had higher IgG titers two months after recovery[11,12]. In contrast, Meitian Yan et al.[13] did not find any difference in the rate of positive IgG antibodies in the comorbidity group.

### Limitations

This study has some limitations. ELISA tests can lead to false-positive results due to cross-reactivity with antibodies against other seasonal human coronaviruses[14]. To better interpret the results, it is necessary to validate the findings with larger case series. In addition, having the PCR results of people who were vaccinated in Group I could, of course, strengthen our results further.

## CONCLUSIONS

IgG antibody seropositivity was found to be 95.5% in people who did not have COVID-19 and received CoronaVac vaccination. It was found that COVID-19 IgG antibody levels decreased with increasing age and BMI. Antibody levels were found to be higher in health care workers working in environments with high viral load compared with others. The presence of comorbidities (i.e., DM and CVD) decreased COVID-19 IgG antibody levels. As a result of this study, new information has emerged that will shed light on future studies with larger case series. It is evident that vaccination doses and repetitions should be reviewed especially in terms of age, obesity, and comorbidities.

## AUTHORS' CONTRIBUTION

**EÇG:** Conceptualization, Formal analysis, Writing – original draft. **AÇ:** Data curation, Formal analysis, Writing – original draft. **BE:** Data curation, Formal analysis. **NS:** Data curation.

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# COVID-19 findings in chest computed tomography

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## SUMMARY

**OBJECTIVE:** The aim of this study was to describe chest computed tomography image findings in patients with COVID-19.

**METHODS:** The chest computed tomography scans of 453 hospitalized patients with confirmed COVID-19 were collected at two tertiary care Brazilian hospitals. Demographics and clinical data were extracted from the electronic record medical system.

**RESULTS:** The main chest computed tomography findings were ground-glass opacities (92.5%), consolidation (79.2%), crazy-paving pattern (23.9%), parenchymal bands (50%), septal thickening (43.5%), and inverted halo sign (3.5%). Of the 453 hospitalized patients, 136 (30%) died. In this group, ground-glass opacities (94.1%), consolidation (89.7%), septal thickening (58.1%), crazy-paving pattern (52.2%), and parenchymal bands (39.7%) were the most common imaging findings.

**CONCLUSIONS:** In a dynamic disease with a broad clinical spectrum such as COVID-19, radiologists can cooperate in a better patient management. On wisely indicated chest computed tomography scans, the fast identification of poor prognosis findings could advise patient management through hospital care facilities and clinical team decisions.

KEYWORDS: COVID-19. CT x ray. Lung. Thorax. SARS-CoV-2.

## INTRODUCTION

Being declared by the World Health Organization (WHO) as a pandemic on March 11, 2019, the novel coronavirus disease (COVID-19)[1] became a major challenge for health care systems worldwide. It was first described on December 2019 as an "unknown etiology pneumonia" at Hubei, a province of China[2]. COVID-19 will hallmark human history as one of the most impressive public health calamities: a life-threatening disease steadily increasing the cause of more than 1 million death and 141 million confirmed cases worldwide[3], not to mention the social, economic, and political distress.

In this scenario, thoracic imaging can be assessed for diagnosis and clinical course monitoring. Although X-rays and ultrasonography have proved their utility, especially on intensive care unit (ICU) and campaign hospitals settings, high-definition computed tomography (HDCT) is the first choice when imaging is required due to its high sensitivity[4-6]. However, the low specificity of the computed tomography (CT)[7] explained why radiology societies' recommendations are against the method as an initial approach to diagnosis[8,9]. Even though reverse transcription polymerase chain reaction (RT-PCR) tests were adopted as the reference standard, dissociation between laboratory and tomographic findings must be expected and CT manifestations remain critical for patient management when first RT-PCR results are negative or unknown[10].

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To contribute with the current literature, the present study describes chest CT findings in patients with COVID-19 in the Brazilian State of Espírito Santo and correlates the results with in-hospital mortality.

## METHODS

A retrospective study was conducted focusing on analyzing thoracic findings in hospitalized patients with SARS-CoV-2 confirmed with real-time reverse transcriptase polymerase chain reaction (rRT-PCR) of respiratory secretions or rapid detection tests to detect the SARS-CoV-2 antibody. It was a multicentric data analysis by two tertiary care Brazilian hospitals, one in Vitória-ES (Hospital Universitário Cassiano Antônio Moraes or HUCAM) and the other one in Serra-ES (Hospital Estadual Jayme Santos Neves or HEJSN).

All imaging examinations were performed according to the clinical indications. CT imaging was performed using Toshiba Alexion 16-slice (Toshiba Medical Systems, Nasu, Japan) at HEJSN and Toshiba 64-slice (Toshiba Medical Systems) at HUCAM. The protocols in both institutions were similar with 2-mm-section thickness, 40 cm field of view, 120 kV (peak), and 200–260 mA. This study was approved by the research ethics committee (CAAE 31424720.1.0000.5071).

Four radiologists (C.C., Y.O., B.B.Z., and W.B. with 1, 3, 3, and 5 years of experience in interpreting CT images, respectively) reviewed the first CT scans of these patients. The radiologists evaluated the initial CT for ground-glass opacities, consolidation, septal thickening, parenchymal bands, inverted halo sign, crazy-paving pattern, lobes affected, pleural effusion, thoracic lymphadenopathy (lymph node size of ≥1 cm in short-axis dimension), and other abnormalities (pneumothorax, pneumomediastinum, bronchiectasis, pulmonary cysts, cavitated nodule, pulmonary emphysema, and random nodules). They classify the degree of lung involvement qualitatively through visual analysis as none (0%), minimal (1–25%), mild (26–50%), moderate (51–75%), or severe (76–100%). Clinical information was collected from the electronic medical records. The analysis of the axial distribution of the lesions was performed, which was classified as lesions with central distribution or lesions with peripheral distribution.

## RESULTS

In this study, 453 CTs of hospitalized patients from March to July 2020 were analyzed. The main CT findings were ground-glass opacities in 419 (92.5%) patients, consolidation in 359 (79.2%), crazy-paving pattern in 122 (23.9%), parenchymal bands in 226 (50%), septal thickening in 197 (43.5%), and inverted halo sign in 16 (3.5%) patients. Pleural effusion was found in 83 (18.3%) patients and lymph node enlargement in 26 (5.7%), as given in Table 1.

We observed severe pulmonary involvement in 59 (13%) patients, moderate involvement in 145 (32%), mild involvement in 135 (29.8%), minimal involvement in 95 (21%), and 19 (4.2%) with none pulmonary involvement. The qualitative visual assessment of each one of the pulmonary lobes revealed a

Table 1. Demographics and CT characteristics.

| | Total (n) | In-hospital mortality |
|---|---|---|
| Number of patients | 453 | 136 |
| Age (year) Mean=60.14±17.4, n (%) | | |
| ≤20 | 5 (1.1) | 0 (0) |
| 21–39 | 63 (13.9) | 5 (3.7) |
| 40–59 | 141 (31.1) | 34 (25) |
| 60–79 | 191 (42.2) | 70 (51.5) |
| 80–99 | 53 (11.7) | 27 (19.9) |
| Sex, n (%) | | |
| Female | 193 (42.6) | 57 (41.9) |
| Male | 260 (57.4) | 79 (58.1) |
| Pulmonary involvement, n (%) | | |
| 0% | 19 (4.2) | 5 (3.7) |
| 1–25% | 95 (21) | 13 (9.6) |
| 26–50% | 135 (29.8) | 25 (18.4) |
| 51–75% | 145 (32) | 53 (39) |
| 76–100% | 59 (13) | 40 (29.4) |
| CT findings, n (%) | | |
| Ground-glass opacity | 419 (92.5) | 128 (94.1) |
| Consolidation | 359 (79.2) | 122 (89.7) |
| Parenchymal bands | 226 (50) | 54 (39.7) |
| Septal thickening | 197 (43.5) | 79 (58.1) |
| Crazy-paving pattern | 122 (23.9) | 71 (52.2) |
| Pleural effusion | 83 (18.3) | 29 (21.3) |
| Lymph node enlargement | 26 (5.7) | 12 (8.8) |
| Pulmonary emphysema | 17 (3.8) | 6 (4.4) |
| Inverted halo | 16 (3.5) | 3 (2.2) |
| Bronchiectasis | 14 (3.1) | 3 (2.2) |
| Pneumothorax | 10 (2.2) | 2 (1.5) |
| Tree-in-bud | 8 (1.8) | 1 (0.7) |
| Pulmonary cysts | 7 (1.5) | 2 (1.5) |

predominance of greater involvement in the lower lobes. Most patients had a bilateral multilobar pattern of involvement, with only nine patients with unilobar commitment.

Out of 453, 136 (30%) patients died during hospitalization. Of these, the distribution of the findings occurred as follows: ground-glass opacities in 128 (94.1%) patients, consolidation in 122 (89.7%), crazy-paving pattern in 71 (52.2%), septal thickening in 79 (58.1%), parenchymal bands in 54 (39.7%), and inverted halo sign in three (2.2%). Pleural effusion was found in 29 (21.3%) patients, being the sole observation in one of them (0.7%). Lymph node enlargement was identified in 12 (8.8%) individuals who died and 5 (3.7%) had normal initial CT. Pneumomediastinum was found in six patients, five of whom died (Figures 1 and 2).

Of the patients who died, 13 (9.6%) had minimal pulmonary involvement, 25 (18.4%) had mild involvement, 53 (39%) had moderate involvement, and 40 (29.4%) had severe involvement.

## DISCUSSION

The pulmonary pathogenesis caused by SARS-CoV-2 has been associated with the coronavirus entrance in cells through angiotensin-converting enzyme two receptor, abundant on the

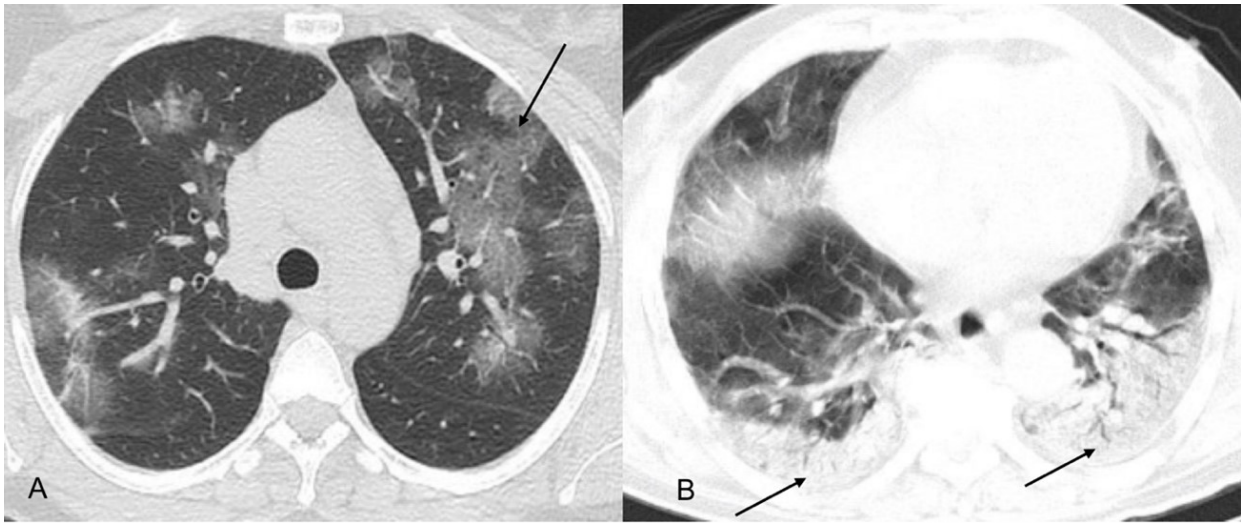


Figure 1. (A) Opacities with ground-glass attenuation (arrow). (B) Diffuse consolidation (arrows).

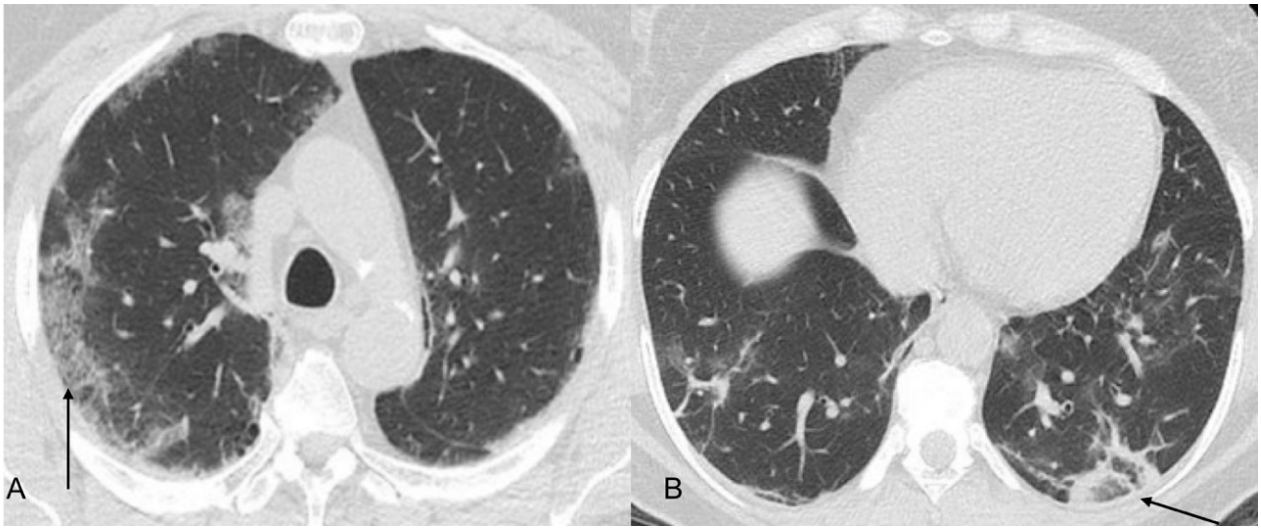


Figure 2. (A) Reticular opacities and ground-glass attenuation (arrow). (B) Ground-glass attenuation opacities surrounded by a consolidation ring – the reversed halo sign (arrow).

acinar side of pneumocytes within alveolar spaces. Once tissues are perpetrated, immune and nonimmune cells promote cytokines storm, resulting in damage to the host[11]. Fever and respiratory symptoms are the most common manifestations, as expected for an infectious pulmonary disease. Clinical findings can be diverse, ranging from asymptomatic patients to critical disease, and from sole lung impairment to multisystemic presentations[11,12].

Although mild and moderate presentations are preponderant, COVID-19 is a dynamic disease with a quick clinical deterioration among severe symptomatic individuals, a group with considerable risk of prolonged critical illness and death[13]. Mortality among hospitalized patients in ICUs varies from 30% to 70% in the literature[14]. Similarly, we found 30% mortality in our patients, with the most important findings related to mortality: age over 60 years, female, and moderate or severe pulmonary involvement. To avoid unwanted outcomes, physicians must identify the main factors of poor prognosis, making quicker decisions and setting appropriate treatment goals[13]. An extent of different scenarios must be considered while interpreting deaths, from the environmental factors to the therapeutic limitations. In addition to this complexity, the results of the present study have important practical implications, providing indicators related to the increased risk of death from COVID-19 and meaningful on guiding physicians' decisions about intensive care practices in ICU settings[14].

Qualitative visual assessment of pulmonary involvement is a metric worth attention. In this study, most of the hospitalized patients had a pulmonary involvement more than 25% and the majority of the patients who died had pulmonary involvement more than 50%, inferring a worse prognosis related to the size of the affected area. Toussie et al.[15] reported that patients with ground-glass opacities represented in at least two pulmonary zones are more likely to need hospitalization and those in three zones were more likely to intubate, reinforcing the prerogative that the largest area affected is related to worse prognosis.

The main CT findings are similar to those that have been described in the literature, including predominantly bilateral multifocal ground-glass opacities, sometimes associated with a superimposed thin septal thickening ("crazy-paving" pattern), usually involving several pulmonary lobes and with distribution predominantly peripheral in the parenchyma[16,17].

Pulmonary cavitation and pneumothorax are less common findings in COVID-19 and, when described, should raise suspicion for other potential causes[11]. The low incidence of pleural effusion and the absence of other findings, such as lymph node enlargement, nodules, and excavated lesions, are in line with recent international experience[18]. Unilateral lesions are also uncommon, described only in 2% of patients with COVID-19 in the literature, and may be useful in differentiating COVID-19 pneumonia from other conditions[11].

It is important to recognize that imaging findings of the new coronavirus share some similarities with other diseases that cause viral pneumonia, particularly those within the same viral family (SARS and MERS). As new cases are identified, other exclusive pulmonary manifestations may emerge as potential points for discernment in this patient population. Future research studies will be essential to determine how patients with parenchymal lung disease evolve after the treatment[19]. Vidal et al.[20] reported that bacterial, fungal, and viral coinfections and superinfections in patients hospitalized with COVID-19 are low; however, when present, they can cause serious diseases. Therefore, any unusual finding may lead to further investigation.

A limitation of our study is the lack of data on patients' comorbidities; however, Revsing et al.[11] reported an increased risk of developing severe disease and increased mortality in patients with underlying cardiovascular disease, diabetes mellitus, hypertension, chronic lung disease, cancer (particularly hematological malignancies, lung cancer, and metastatic disease), obesity, and chronic kidney disease. The Centers for Disease Control and Prevention also includes immunocompromised status and liver disease as potential risk factors for serious illnesses, although specific data on the risks associated with these conditions are limited[11,21].

Chest radiography is generally the initial imaging method and the American College of Radiology advises against the use of CT as a first-line tool in the diagnosis of COVID-19, recommending it to be used in moderation and reserved for symptomatic patients hospitalized with specific clinical indications, as an assessment of complications[11]. However, Tao et al.[22] reported that 97% of patients confirmed with COVID-19 with RT-PCR assays had positive results on chest CT, confirming a high sensitivity in detecting the disease.

## CONCLUSIONS

This work represents an investigation of the chest CT findings in hospitalized patients with COVID-19. The radiologist plays a crucial role in the fast identification and early diagnosis of new cases, in addition to identifying factors of worse prognosis, which can be a great benefit not only for the patient but also for the broader health surveillance and response systems public[23]. Therefore, the CT evaluation, when well indicated, offers important information, identifying typical findings or suggesting other diagnoses

and findings about a worse prognosis, unfavorable evolution, or mortality, such as consolidation or crazy-paving pattern, pneumomediastinum, and moderate/severe pulmonary involvement.

## AUTHORS' CONTRIBUTIONS

**CC:** Conceptualization, Data curation, Formal analysis, Writing – original draft, Writing – review & editing. **FFF:** Conceptualization, Data curation, Formal analysis, Writing – original draft, Writing – review & editing. **LL:** Conceptualization, Data curation, Formal analysis, Writing – review & editing. **RMB:** Conceptualization, Data curation, Formal analysis, Writing – review & editing. **BBZ:** Conceptualization, Data curation, Formal analysis, Writing – review & editing. **YOJ:** Conceptualization, Data curation, Formal analysis, Writing – review & editing. **WHB:** Conceptualization, Data curation, Formal analysis, Writing – review & editing.

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# Decreased ovarian reserve and ovarian morphological alterations in female rat offspring exposed to a ketogenic maternal diet

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## SUMMARY

**OBJECTIVE:** This study evaluates the effects of a ketogenic diet on morphology and follicle reserve.

**METHOD:** Sixteen Sprague-Dawley rats were randomized into two groups: standard diet group (n=8) and ketogenic diet group (n=8). Rats were time mated. Dams were permitted to deliver spontaneously. The animals were monitored for the onset of puberty. All the rats were weighed and anesthetized, serum anti-Müllerian hormone level was measured, and the oviducts were removed. The morphological characteristics of follicles were determined and total ovarian volumes were calculated.

**RESULTS:** The mean ovarian volume was statistically significantly lower in the ketogenic diet group compared to the standard diet group (14.41±0.99 $mm^3$ *versus* 18.89±1.28 $mm^3$) (p=0.000). The mean number of antral follicles was 13.63±1.80 in the standard diet group and 4.462±0.760 in the ketogenic diet group. The mean ovarian weight of the ketogenic diet group was significantly lower than that of the standard diet group (0.42±0.06 g *versus* 0.815±107 g). The mean anti-Müllerian hormone levels were significantly higher in the standard diet group compared to the ketogenic diet group (1.023±4.75 ng/mL *versus* 0.69±0.07 ng/mL) (p=0.000). The mean percentage of staining of Ki-67 was 35.28±4.75 in the standard diet group and 16.98±3.33 in the ketogenic diet group (p=0.000).

**CONCLUSION:** Maternal ketogenic diet reduces ovarian follicular reserve in female offspring and has important implications for maintaining reproductive potential at a population level.

KEYWORDS: Ketogenic diet. Ovarian reserve. Anti-Mullerian hormone. Reproduction.

## INTRODUCTION

Epidemiological and experimental studies show that nutrition and environmental conditions in early life affect development in later life. It has been shown that the mother's nutritional status during pregnancy affects the reproductive potential of the female offspring[1].

Although an inadequate or inappropriate pregnancy diet causes permanent harmful effects on maternal and fetal metabolism, this results in the changes in fetal physiology[2]. Gestational diets may alter fetal metabolism, and physiology can affect organ development and function[3].

The maternal gestational diet has been shown to affect numerous parameters of offspring reproductive function, including follicular reserve, ovarian vascularity, and estrus cycle in rat models[4].

Ketogenic diets (KD) are diets with high-fat, low-carbohydrate content which have effective management of various diseases such as epilepsy and obesity[5]. Physiological ketosis might be beneficial in improving obesity, type 2 diabetes, and cardiovascular disease risk factors[6]. It has been reported that the KD also has effects on polycystic ovary syndrome, which

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is an important endocrine problem in gynecology and the risk of type 2 diabetes increases in the follow-up[7].

However, it has been shown that a high-fat diet (HFD), which has similar fat content to KD, can adversely affect obesity, follicular growth, and development in mice[8].

Previous studies of gestational ketosis have focused on maternal ketosis caused by malnutrition, prolonged hunger, or diabetes[9]. However, these conditions and their consequences are different from stable ketosis resulting from the consumption of KD.

We compared the differences in the number of primordial, primary, secondary, and antral follicles; cycle start days; ovarian volume; anti-Müllerian hormone (AMH) levels; and Ki67 of female offspring of rats fed with the SD and KD during preconception and pregnancy period. Evaluation of the main components of reproductive system functioning was performed by examining the ovaries and ovarian follicles, in order to reveal the influence in the offspring.

# METHODS

## Ethics and animals

The study was conducted in Sakarya University's SÜDETAM laboratory under the authority of Sakarya University's experimental animal ethics committee on November 4, 2020, under decision nº. 62. According to the "The European Commission Directive 86/609/ECC guideline" protocol, applications for all research animals were carried out.

A total of 16 Sprague-Dawley rats (weight 200–250 g; age 65–75 days) were kept under a 12-h light/dark cycle and at a constant temperature of 22±2°C with food and water available ad libitum. Rats were randomized into two groups: standard diet (SD) group (n=8) and KD group (n=8).

Both diets were manufactured by Arden Research & Experiment, Ankara, Turkey. SD consists of 5% fat, 76.1% carbohydrate, and 18.9% protein. KD consists of 67.4% fat, 0.6% carbohydrate, and 15.3% protein.

Rats were time mated, then housed individually in standard rat cages with free access to water at a constant temperature maintained at 25°C and a 12-h light/dark cycle. Dams were permitted to deliver spontaneously. On the day of birth, the number of female rats was 35 in the SD group and 26 in the KD group.

At the beginning on PND 28, the animals were monitored daily for the vaginal opening, a physical marker of the onset of puberty.

All the rats were anesthetized by an intramuscular administration of 50 mg/kg ketamine hydrochloric acid (Ketalar; Eczacibasi Warner-Lambert, Istanbul, Turkey) and 7 mg/kg xylazine hydrochloric acid (Rompun; Bayer, Istanbul, Turkey). They were decapitated immediately. The blood samples were collected to measure the serum AMH levels. The aseptic technique was used to make a ventral midline incision expose the reproductive organs, and the oviducts were removed.

Previously fixed ovaries were embedded in paraffin, cut into 5 µm, and stained with hematoxylin and eosin (HE). Histological assessments were performed by the same pathologist, who was blinded to the study conditions (Figures 1A and 1B).

The morphological characteristics of follicles were determined according to the previous report as follows[10,11]:

- Primordial follicle, a single oocyte surrounded by a monolayer of squamous cells;
- Primary follicle, an oocyte without antrum surrounded by a monolayer or more than one cuboid or prismatic cell;
- Secondary follicle, consisting of a single oocyte and a single antrum;
- Antral follicle, consisting of a single oocyte and enlarged antrum;
- Corpus luteum, consisting of large volumes of luteal cells surrounded by capillary blood vessels[10,11].

## Histological preparation for volume measurement

Histological sections were stained with HE and then examined under an inverted microscope (Nikon Eclipse-TI, USA). Ovarian sections were photographed digitally at 4× magnification using a reference ruler.

## Volume calculation

ImageJ software was used (http://www.rsb.info.nih.gov/ij/download) in order to measure the images obtained from histologically prepared sections. Total ovarian volumes were calculated using the following formula from pictures obtained from the consecutive histological sections:

$V_{ovary} = tx\ Px\ \Sigma^{N}_{i=1}Ai$[12]

## Immunohistochemical analysis

Tissue samples to be made immunohistochemical (IHC) analysis for Ki-67 immunoreactivity evaluation, a light microscope at 200× magnification (Nikon Eclipse Ni invert microscope) was examined (Figures 1C and 1D). A scoring system has used the protocol reported by Panzan et al.[13] Immunoexpression was evaluated at 400× magnification using the semiquantitative method.

## Hormonal assays

AMH was quantitatively estimated in rat serum samples using enzyme-linked immunosorbent assay (ELISA) kits (My

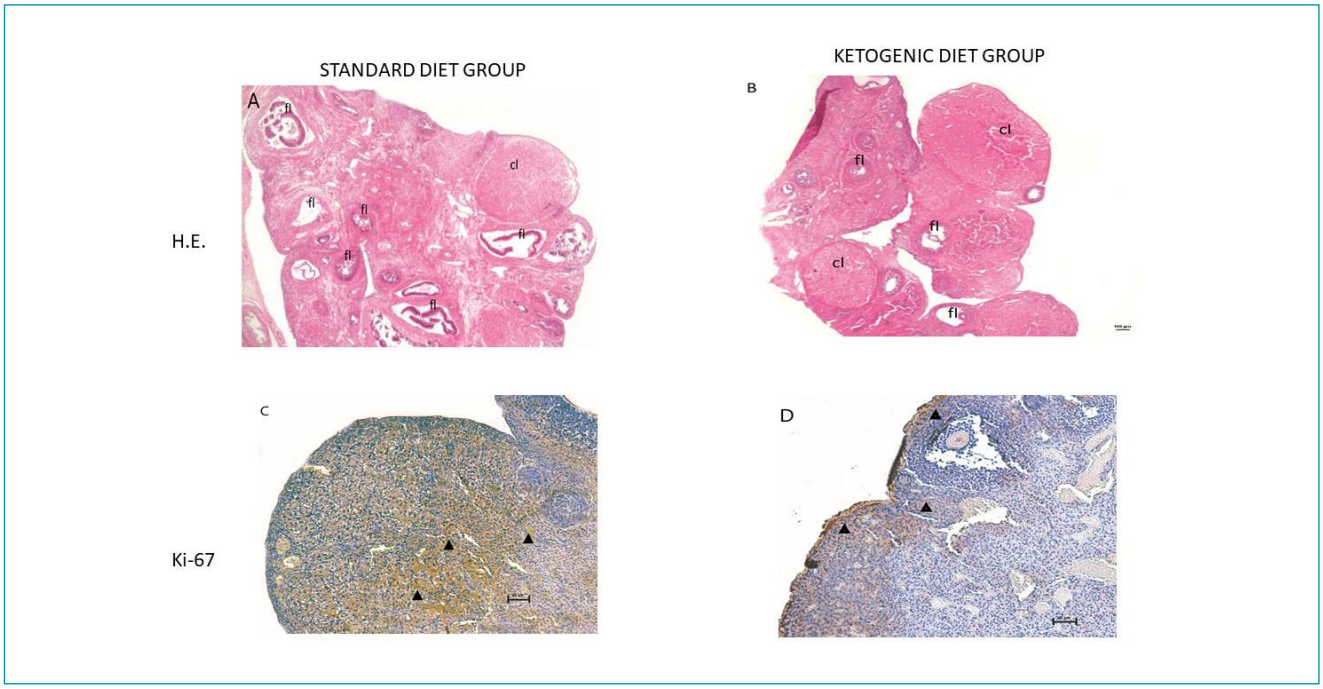


Figure 1. Fl: follicles; CL: corpora lutea. Standard and ketogenic diet ovarian Hematoxylin and eosin stain (HE) and Ki-67 immunohistochemistry pictures, 40x100 scale bar. The number of follicles was higher in the Standard diet group (A) compared to the ketogenic diet group (B). Ki-67 immunohistochemistry positivity was observed to be more intense in the Standard diet group (C) compared to the ketogenic group (D); brown-stained cells are considered positive (black arrowhead).

BioSource, Rat AMH ELISA Kit catalog no: MBS2509909, San Diego, CA, USA).

### Statistical analysis

Statistical analyses were performed using the SPSS 24.0 package program (SPSS Inc. and Lead Tech. Inc., Chicago, IL, USA). The variables were investigated using visual and the analytical methods Kolmogorov–Smirnov test to determine whether they are normally distributed. Mann-Whitney U test was used for the non-normally distributed numerical data. While investigating the associations between non-normally distributed variables, the correlation coefficients and their significance were calculated using the Spearman test. Results are given as mean±standard deviation. For all statistical analyses, a two-tailed $p<0.05$ was considered statistically significant.

## RESULTS

In our study, we obtained 35 female offspring in the SD group and 26 in the KD group. We found the mean ovarian volume of 18.89±1.28 $mm^3$ in the SD group and 14.41±0.99 $mm^3$ in the KD group (p=0.000) (Figure 2A).

The mean number of primordial follicles was 106.94±4.57 in the SD group and 59.54±4.13 in the KD group (p=0.000) (Figure 2B).

The number of primary follicles was 62±4.04 in the SD group and that in the KD group was decreased to 31.03±3.79 (p=0.000) (Figure 2C).

The mean number of secondary follicles was 33.34±3.39 in the SD group and 11.77±1.84 in the KD group (p=0.000) (Figure 2D).

The mean number of antral follicles was 13.63±1.80 in the SD group and 4.462±0.760 in the KD group (p=0.000) (Figure 2E). The mean corpus luteum count was 7.54±0.78 in the SD group and 2.038±0.662 in the KD group (Figure 2F).

The mean ovarian weight of the SD group was significantly lower than that of the KD group (0.815±107 g *versus* 0.42±0.06 g) (p=0.000) (Figure 2G).

The mean AMH levels were significantly higher in the SD group compared to the KD group (1.023±4.75 ng/mL *versus* 0.69±0.07 ng/mL) (p=0.000) (Figure 2H).

The mean percentage of staining of Ki-67 was 35.28±4.75 in the SD group and 16.98±3.33 in the KD group (p=0.000) (Figure 2I).

The correlation between the ovarian volumes and the number of primordial follicles, AMH levels, and Ki-67 staining percentages shows no statistically significant difference between the SD and KD groups.

The results were as follows: for KD group, the ovarian volumes and the number of primordial follicles (p=0.676, r=0.086),

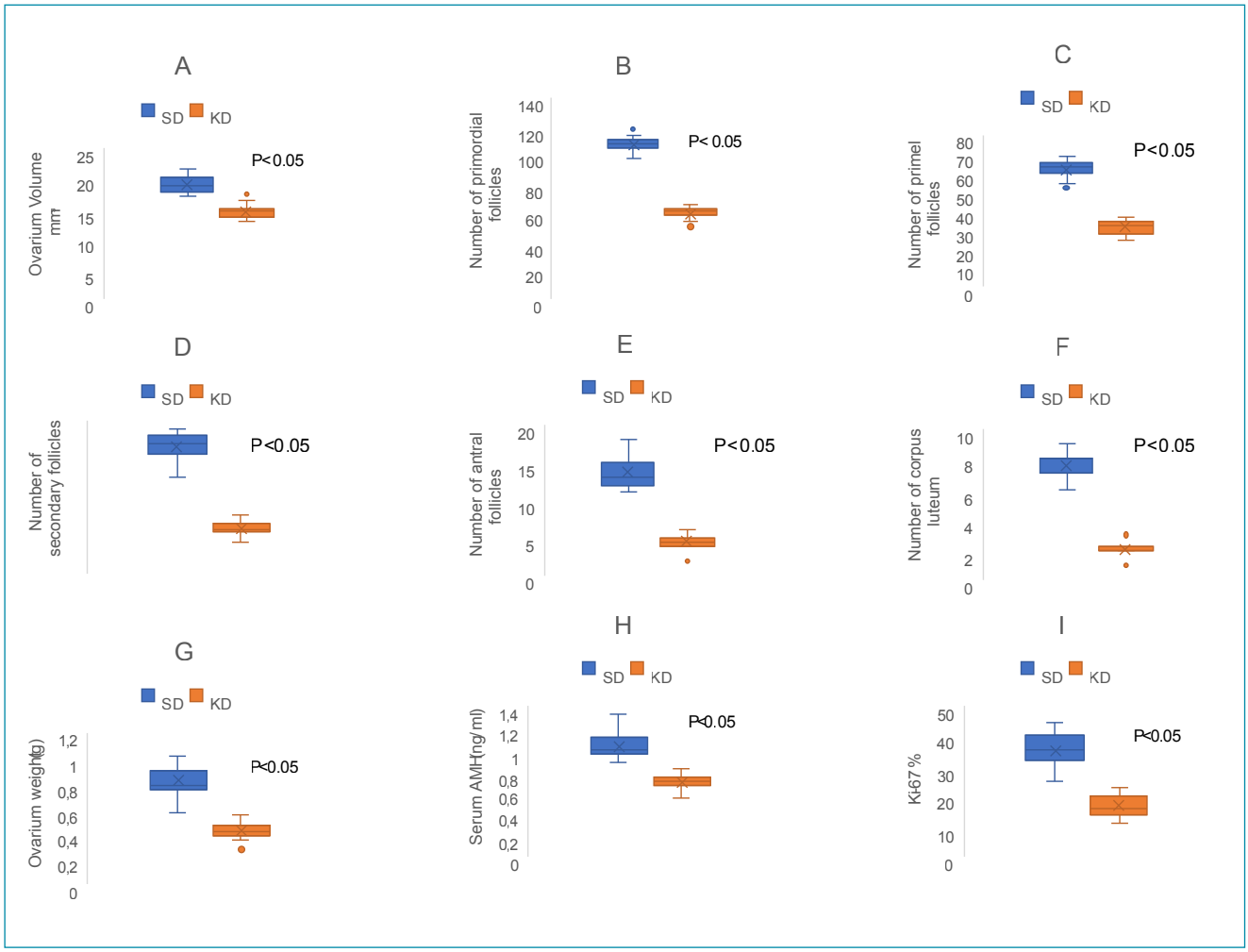


Figure 2. (A) Comparison of ovarian volume; (B) and comparison of number of primordial primary; (C) secondary; (D) antral; (E) number of corpus luteum; (F) ovarian weight; (G) serum AMH concentrations; (H) and Ki-67 staining percentages; (I) in standard diet and ketogenic diet groups. Box plot shows the comparison of standard diet and ketogenic diet (standard diet n=35; ketogenic diet n=26).

Ki-67 concentration (p=0.229, r=0.244), and AMH (p=0.312, r= -0.206). For SD group, the ovarian volumes and the number of primordial follicles (p=0.688, r= -0.070), Ki-67 concentration (p=0.344, r= -0.165), and AMH (p=0.803, r= -0.044).

## DISCUSSION

This study shows that maternal gestational KD leads to follicular defects occurring during fetal life in oocyte and follicular development offspring. Pregnancy is the critical window of fetal ovarian vulnerability of mothers' offspring and has shown a loss of oocyte-derived follicular growth factors and increased oxidative stress levels[14]. Exposure to a suboptimal environment during fetal and early postnatal development has to impair the programming of offspring ovarian reserve in animal models[15].

Although we know that the KD exhibits anti-inflammatory properties[16], our experiments demonstrate the adverse effects of the maternal KD on the follicular count of offspring. These adverse effects in our study were similar to the findings found due to studies using HFDs[17]. KD, which is similar to HFD in content, affects ovaries similar to HFD.

It is known that maternal obesity due to HFD seriously affects offspring phenotype by altering fetal programming in various systems, including reproduction[18]. Our study observed these results, which developed due to obesity caused by HFD, without resulting in obesity in KD.

Pubertal timing and ovarian function in female rats are affected by maternal calorie restriction and maternal high-fat nutritional status[19]. Studies on HFD have shown the adverse effects of obesity on oocyte count, quality and maturity, fertilization rate, and subsequent embryo quality[20]. Connor et al.[21]

found that pups from high-fat-fed dams exhibit early puberty and irregular estrous cycles by having prolonged and persistent estrus. It has been shown that reproductive function parameters related to steroid synthesis, including pubertal age, affected rat offspring that received an HFD[21].

Our study found that puberty occurred earlier in the KD group. All these data indicate that poor quality of nutrition can disrupt ovarian function, especially the development and quality of oocytes.

Expression of the Ki-67 antigen, indicating the proliferative potential of cells, is expressed in all active stages of the cell cycle (G1, S, G2, and mitosis)[22]. The expression of Ki-67 was more apparent in SD follicles than in the KD group. Primordial follicle activation requires a signaling system including cytokines and growth hormones that mediate intercellular communication[23]. This suggests that primordial follicle development due to KD is negatively affected by this pathway.

Minge et al.[20] found that mice fed an HFD had delayed embryonic development[20]. Similarly, using mice, Aiken et al.[4] found no difference in ovarian weight between the two groups. Our study observed that the ovarian volume and ovarian weight decreased in the KD group without obesity. This situation suggests that local side effects occur on the ovarian tissue without systemic effects such as excessive weight gain in the KD group. The significantly lower serum AMH level in the KD group than the SD group seems to be compatible with histopathological examination. The mean number of primordial, primary, secondary, antral, and the number of corpus luteum in the KD group displayed a statistical significance difference compared with the SD group. It is known that rat studies cannot fully reflect human physiology. In this case, this constitutes the main limitation of our study.

## CONCLUSION

The maternal KD may reduce ovarian follicular reserve in offspring. The model may be helpful in future studies providing further insight into the pathophysiology of human reproduction in the setting of the use of KD.

## AUTHORS' CONTRIBUTIONS

**ÖB:** Conceptualization, Formal analysis, Investigation, Methodology, Project administration, Resources, Supervision, Visualization, Writing – original draft, Writing – review & editing. **MSB:** Formal analysis, Methodology, Validation, Visualization, Writing – original draft, Writing – review & editing. **EK:** Data curation, Formal analysis, Funding acquisition, Resources, Software. **VT:** Data curation, Funding acquisition, Resources, Software.

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# Can blood urea Nitrogen-to-Albumin ratio predict mortality in patients with moderate-to-severe COVID-19 pneumonia hospitalized in the intensive care unit?

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## SUMMARY

**OBJECTIVE:** Many laboratory parameters allow to follow up the course of the disease and reveal its clinical severity, particularly in patients with coronavirus disease 2019 (COVID-19) pneumonia. In this study, we aimed to investigate the role of the blood urea nitrogen-to-albumin ratio in predicting the mortality in COVID-19 patients with moderate-to-severe disease who are hospitalized in the intensive care unit.

**METHODS:** A total of 358 patients who were hospitalized in intensive care unit at our hospital between November 1, 2020 and May 15, 2021 were included in this study. During their course of intensive care, surviving patients were included in Group 1 and nonsurviving patients in Group 2.

**RESULTS:** There were no statistically significant differences between the two groups in terms of gender, smoking, and chronic obstructive pulmonary disease rates. In multivariate logistic regression analysis, advanced age (OR 1.038, 95%CI 1.014–1.064, p=0.002), neutrophil-to-lymphocyte ratio (OR 1.226, 95%CI 1.020–1.475, p=0.030), blood urea nitrogen-to-albumin ratio (OR 2.693, 95%CI 2.019–3.593, p<0.001), and chest computed tomography severity score (OR 1.163, 95%CI 1.105–1.225, p<0.001) values were determined as independent predictors for in-hospital mortality.

**CONCLUSION:** In this study, we showed that the blood urea nitrogen-to-albumin ratio, which was previously shown as a predictor of mortality in patients with various pneumonia, was an independent predictor of mortality in patients with COVID-19 pneumonia.

KEYWORDS: COVID-19. Pandemic. Inflammation. Mortality. Intensive care.

## INTRODUCTION

Besides respiratory and gastrointestinal problems, coronaviruses may also cause neurological and visceral organ damage. The coronavirus disease 2019 (COVID-19), which emerged in Wuhan, China, at the end of 2019, caused a pandemic[1]. Due to this, millions of people lost their lives, and the entire world is affected for more than a year[2].

With the COVID-19 pandemic, it has become especially important to predict morbidity and mortality in patients. Treatment of vulnerable groups, such as those with moderate and severe disease, will play a key role in the management of the "health crisis" caused by the pandemic. Many laboratory parameters, such as C-reactive protein, fibrinogen, troponin, ferritin, and Dd-dimer, allow to follow up the course of the disease and reveal its clinical severity, particularly in patients with COVID-19 pneumonia[3]. One of the most important of these is albumin. Synthesized by the liver, it plays an important

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role in maintaining the osmotic pressure as well as in the transport of many vital substances[4]. In a study, it was shown that it may be a predictor of mortality in hospitalized COVID-19 patients[5]. In addition, the amount of urea absorbed by the kidneys causes an increase in blood urea nitrogen (BUN) levels. This can also show dehydration, a common condition among patients with pneumonia, and indicate a poor prognosis[6]. In the light of this information, the ratio of blood urea nitrogen-to-albumin (B/A) appears to be an important prognostic marker. In a recent study, it was shown that it may be a predictor of mortality in patients with aspiration pneumonia[7].

In this study, we aimed to investigate the role of the B/A ratio in predicting mortality in COVID-19 patients with moderate-to-severe disease who are hospitalized in the intensive care unit (ICU).

## METHODS

Patients with moderate and severe COVID-19 pneumonia hospitalized in the ICU between November 1, 2020 and May 15, 2021 were consecutively included in this study. The real-time polymerase chain reaction (RT-PCR) tests of nasal and pharyngeal swab samples of all patients included in the study were found positive. Demographic data of all patients (e.g., age, the presence of hypertension, diabetes mellitus, coronary artery disease) and laboratory parameters at the time of admission were noted. Intubated patients admitted to the ICU and patients with critical disease, malignancy, known systemic inflammatory disease, liver failure, and serum creatinine values above 2 mg/dL were excluded from the study. Involvement rates were calculated by evaluating the thorax tomography images of all patients at the time of admission. During their course of intensive care, surviving patients were included in Group 1 and non-surviving patients in Group 2.

Indications for hospitalization in the ICU of all patients were in line with the recommendations of the scientific committee of our country[8]. Chest Computed Tomography Severity Score (CT-SS) scoring, which was developed by Yang et al.[9], was utilized to assess chest CT images.

### Statistical analysis

In this study, SPSS version 21.0 (IBM Statistical Package for the Social Sciences Statistic Inc., Chicago, IL, USA) program was utilized to analyze the data. "Kolmogorov-Smirnov test and Shapiro-Wilk test" were used for normality distribution analysis. Student's *t* test was used for the data presenting normal distribution and Mann-Whitney U test for those that did not conform to normal distribution. These data were shown as mean±standard deviation or as mean (interquartile range, 25th percentile-75th percentile). Categorical variables were shown as frequency and percentage, and "chi-square test" was used for analysis. Multivariate binary logistic regression analysis was utilized to analyze mortality predictors. A $p<0.05$ was accepted statistically significant. In predicting in-hospital mortality, receiver operating characteristics (ROC) curve analysis was performed in order to calculate neutrophil-to-lymphocyte ratio (NLR), CT-SS, B/A ratio, and area under the curves (AUCs). Spearman correlation analysis was utilized to assess a possible linear association between B/A ratio and CT-SS.

## RESULTS

A total of 358 patients were included in the study. Those who did not develop in-hospital mortality were included in Group 1 (n=209, median age=48 [39–61.5] years) and those who did were in Group 2 (n=149, median age=66 [50.5–77] years). There were no statistically significant differences between the two groups in terms of gender, smoking, and chronic obstructive pulmonary disease rates. Age, diabetes mellitus, hypertension, and coronary artery disease rates were significantly higher in Group 2 compared to Group 1 ($p<0.001$, $p=0.002$, $p=0.030$, and $p<0.001$, respectively). Also admission CT-SS of the patients was higher in group 2 ($p<0.001$). Demographic characteristics of all patients are presented in Table 1.

Preoperative blood values of the patients are provided in Table 1. The two Groups were similar in terms of white blood cell and platelet values. In Group 2, while neutrophil counts, NLR, ferritin, troponin I, D-dimer, fibrinogen, C-reactive protein, blood urea nitrogen, creatinine, and B/A ratio values were significantly higher ($p<0.001$, $p<0.001$, $p<0.001$, $p<0.001$, $p=0.001$, $p=0.006$, $p<0.001$, $p<0.001$, $p<0.001$, and $p<0.001$ respectively), hemoglobin, lymphocyte, and albumin values were significantly lower ($p=0.009$, $p<0.001$, and $p<0.001$, respectively).

Multivariate logistic regression analysis was performed to evaluate the predictive value of certain parameters in terms of in-hospital mortality. In this analysis; advanced age (OR 1.038, 95%CI 1.014–1.064, $p=0.002$), NLR (OR 1.226, 95%CI 1.020–1.475, $p=0.030$), B/A ratio (OR 2.693, 95%CI 2.019–3.593, $p<0.001$), and CT-SS (OR 1.163, 95%CI 1.105–1.225, $p<0.001$) values were determined as independent predictors for in-hospital mortality (Table 2).

ROC curve analysis was performed to evaluate B/A ratio, NLR, and CT-SS in predicting mortality. The cutoff value of B/A ratio was 3.4 ( AUC 0.823, 95%CI 0.777–0.870, $p<0.001$, with 74.5% sensitivity and 75.6% specificity) and that of NLR was 2.73 (AUC 0.749, 95%CI 0.696–0.802, $p<0.001$, with 68.5% sensitivity and 70.8% specificity) and CT-SS was 13.5

(AUC 0.754, 95%CI 0.702–0.805, p<0.001, with 63.8% sensitivity and 77.5% specificity) (Figure 1).

There was a mild positive correlation between B/A ratio and CT-SS (r=0.230, p<0.001).

## DISCUSSION

In this study, we showed that the B/A ratio is an independent predictor of mortality in patients with moderate-to-severe COVID-19 pneumonia hospitalized in the ICU. In addition, we found that CT-SS, advanced age, and NLR values were independent predictors of mortality. There was also a mild correlation between CT-SS and B/A ratio.

Serum albumin is an acute-phase reactant with antioxidant properties. It plays a significant role in the destruction of free oxygen radicals synthesized during oxidative stress[10,11]. COVID-19 disease also induces an oxidative stress state in humans, and a study showed that low albumin can predict the severity of the disease[12]. In another study conducted on 319 hospitalized COVID-19 patients, Violi et al.[5] investigated the effect of albumin on mortality. The authors proposed the idea that albumin levels could be used to distinguish COVID-19 patients with elevated mortality risk[5]. Li et al. investigated the effect of albumin on clinical outcomes in 134 COVID-19 patients and found low albumin levels to be significantly associated with pneumonia severity as well as mortality in patients with critical disease[13]. In our study, albumin values were significantly lower in nonsurviving patients.

BUN value is an important indicator of dehydration status and is known to be associated with poor clinical outcomes

Table 1. Demographic features and admission clinic data and laboratory values of the patients.

| | Group 1 n=209 (Survivors) | Group 2 n=149 (Nonsurvivors) | p-value |
|---|---|---|---|
| Age | 48 (39–61.5) | 66 (50.5–77) | <0.001[b] |
| Male/female gender | 82/127 | 66/83 | 0.338[a] |
| Smoking, n (%) | 52 (24.9) | 50 (33.6) | 0.073[a] |
| Hypertension, n (%) | 56 (26.8) | 56 (37.6) | 0.030[a] |
| Coronary artery disease, n (%) | 23 (11) | 39 (26.2) | <0.001[a] |
| Diabetes mellitus, n (%) | 33 (15.8) | 44 (29.5) | 0.002[a] |
| COPD, n (%) | 13 (6.2) | 14 (9.4) | 0.262[a] |
| CT-SS | 11 (9–13) | 17 (12–22) | <0.001[b] |
| WBC ($10^3/mm^3$) | 5.89 (4.66–7.28) | 6.09 (4.66–8.47) | 0.088[b] |
| Hemoglobin (g/dL) | 13.1(11.8–14.1) | 12.6 (11.2–13.7) | 0.009[b] |
| Platelet ($10^3/mm^3$) | 215 (173–253) | 224 (164.5–316.5) | 0.085[b] |
| Neutrophil ($10^3/mm^3$) | 3.4 (2.5–4.4) | 4.3 (3.2–6.2) | <0.001[b] |
| Lymphocyte ($10^3/mm^3$) | 1.7 (1.2–2.2) | 1.1 (0.8–1.4) | <0.001[b] |
| Neutrophil-to-lymphocyte ratio | 2.1 (1.3–2.9) | 3.7 (2.3–6.9) | <0.001[b] |
| Ferritin (mg/L) | 144 (70–295.2) | 275 (135–433.4) | <0.001[b] |
| Troponin I (mg/L) | 3.14 (1–7.49) | 5 (3–13) | <0.001[b] |
| D-Dimer (ng/mL) | 0.53 (0.3–1.04) | 0.81 (0.53–1.36) | 0.001[b] |
| Fibrinogen (mg/dL) | 415 (310.5–550) | 508 (357.5–650) | 0.006[b] |
| Albumin (g/dL) | 3.9 (3.6–4.2) | 3.75 (3.5–4) | <0.001[b] |
| C-reactive protein (mg/L) | 11.3 (3.4–49.6) | 40 (11.6–82.6) | <0.001[b] |
| BUN (mg/dL) | 10.8 (8.9–12.6) | 17.8 (12.5–23.1) | <0.001[b] |
| Creatinine (mg/dL) | 0.77 (0.64–0.96) | 1.03 (0.7–1.39) | <0.001[b] |
| B/A ratio (mg/g) | 2.77 (2.23–3.39) | 4.69 (3.38–6.42) | <0.001[b] |

COPD: chronic obstructive pulmonary disease; CT-SS: Computed Tomography Severity Score; WBC: white blood cell; BUN: blood urea nitrogen; B/A: BUN/albumin. [a]$\chi^2$ test. [b]Mann–Whitney U test. Data are expressed as median and interquartile range (25th–75th percentile).

in patients with heart failure and community-acquired pneumonia[14,15]. The study on 337 COVID-19 patients performed by Liu et al. showed that high BUN values may be associated with mortality[16]. Cheng et al. investigated the importance of BUN and D-dimer values in predicting in-hospital mortality in 305 COVID-19 patients.

BUN values were significantly higher among nonsurvivors (p<0.0001)[17].

In the light of this information about BUN and albumin, an increase in BUN values and a decrease in albumin values, which results in an increased B/A ratio, appear as important prognostic markers. In recent studies, B/A ratio was shown to be a mortality predictor for various diseases[18,19]. In a study on 175 patients with community-acquired pneumonia, Ugajin et al.[10] investigated the effect of the B/A ratio on clinical outcomes and identified the B/A ratio as an independent predictor of ICU need (OR 1.27, 95%CI 1.09–1.47, p=0.002) and mortality (OR 1.10, 95%CI 1.01–1.20, p=0.037)[10]. In a recent study, Ryu et al.[7] investigated the prognostic role of the B/A ratio among 443 patients with aspiration pneumonia and concluded that B/A>7 was an independent predictor of 28-day mortality (OR 3.40, 95%CI 1.87–6.21, p<0.001). In our study, we determined that the B/A ratio is an independent predictor of mortality in patients with COVID-19 pneumonia needing intensive care.

An increase in the neutrophil ratio and a decrease in the lymphocyte ratio, resulting in elevated NLR, play a significant role in the progression and prognosis of various diseases[20,21]. In addition, lymphopenia is the most common hematological finding, seen at a rate of 83% among hospitalized COVID-19 patients[22]. In a meta-analysis of 38 currently published articles, including 5699 patients with severe disease and 6033 nonsurviving patients, high NLR values were shown to be associated

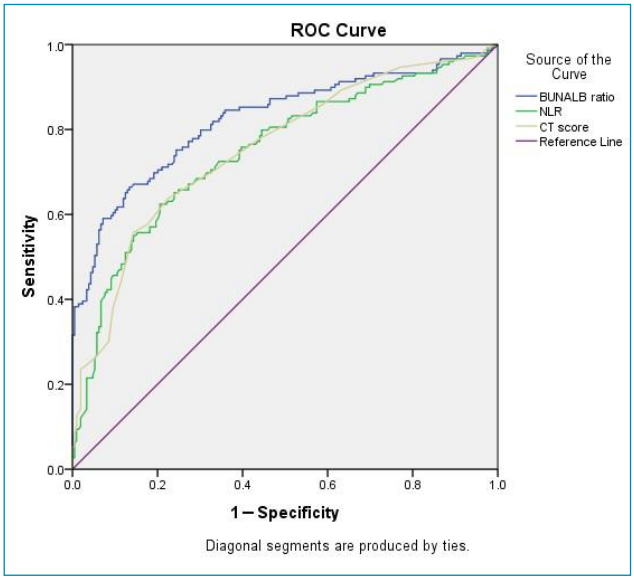


Figure 1. Data of the area under the curve, confidence interval, and cutoff values in receiver operating characteristic curve analysis for blood urea nitrogen-to-albumin ratio (cutoff 3.4, AUC 0.823, 95%CI 0.777–0.870, p<0.001, with 74.5% sensitivity and 75.6% specificity), NLR (cutoff 2.73, AUC 0.749, 95%CI 0.696–0.802, p<0.001, with 68.5% sensitivity and 70.8% specificity) and CT-SS (cutoff 13.5, AUC 0.754, 95%CI 0.702–0.805, p<0.001, with 63.8% sensitivity and 77.5% specificity).

Table 2. Multivariate logistic regression analysis to identify factors affecting in-hospital mortality.

| | Multivariate analysis | | |
|---|---|---|---|
| | p-value | Exp(B) odds ratio | 95%CI Lower–Upper |
| Age | 0.002 | 1.038 | 1.014–1.064 |
| Hypertension | 0.142 | 1.810 | 0.820–3.995 |
| Diabetes mellitus | 0.307 | 0.645 | 0.279–1.494 |
| Hemoglobin | 0.656 | 0.962 | 0.813–1.139 |
| Troponin I | 0.070 | 0.983 | 0.964–1.001 |
| Creatinine | 0.237 | 2.065 | 0.620–6.872 |
| D-Dimer | 0.366 | 1.015 | 0.983–1.047 |
| Fibrinogen | 0.959 | 1.000 | 0.999–1.002 |
| C- reactive protein | 0.170 | 0.995 | 0.987–1.002 |
| Neutrophil-to-lymphocyte ratio | 0.030 | 1.226 | 1.020–1.475 |
| B/A ratio | <0.001 | 2.693 | 2.019–3.593 |
| CT-SS | <0.001 | 1.163 | 1.105–1.225 |

CT-SS: Computed Tomography Severity Score; B/A: blood urea nitrogen/albumin.

with disease severity and mortality[23]. In our study, a high NLR value was an independent predictor of mortality, in line with the literature.

The most important limitation of our study is its single-center and retrospective design. In addition, the number of patients was sparse. Evaluations were made by considering the clinical parameters of the patients at the time of admission to the ICU. These dynamic parameters may change hourly or daily during patient follow-ups. Our study needs to be supported with prospective multicenter studies, including clinical follow-up parameters.

## CONCLUSIONS

This is the first study to show that the B/A ratio, which was previously shown as a predictor of mortality in patients with various pneumonia, was an independent predictor of mortality in patients with COVID-19 pneumonia. In addition, unlike many mortality studies, we calculated the CT-SS values of all patients and showed a slightly positive correlation between the B/A ratio and CT-SS.

## AUTHORS' CONTRIBUTIONS

**FA:** Conceptualization, Data curation, Investigation, Methodology, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. **AKA:** Investigation, Methodology, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. **ME:** Investigation, Methodology, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. **NKK:** Investigation, Methodology, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. **YA:** Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. TT: Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing.

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# Effectiveness of telemedicine in response to the COVID-19 pandemic

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## SUMMARY

**OBJECTIVE:** The aim of this study was to evaluate the utility of the telemedicine care model implemented to treat and guide patients with COVID-19 related symptoms and indicators during the pandemic.

**METHODS:** This is a retrospective study with data collected from the electronic records of standardized forms for assistance. As a way of evaluating the work performed, the number of consultations, types of referrals, efficiency of care, and patient satisfaction were observed.

**RESULTS:** Between April 2 and October 15, 2020, 92 professionals attended 3,660 patients by telemedicine; out of them, 523 (14.3%) were referred to a COVID-19 attending room, 128 (3.5%) to other specialties, 123 (3.4%) to a general emergency department, and 2,886 (78.9%) were monitored via home care. Of the total number of patients, 81 (2.2%) were hospitalized, and 13 (0.35%) died.

**CONCLUSION:** Telemedicine offered useful tools for the care, treatment, and monitoring of patients with COVID-19 during the pandemic. The service was considered by most respondents as satisfactory, resolutive, or safe.

KEYWORDS: Telemedicine. Pandemics. Coronavirus infection. Patient satisfaction.

## INTRODUCTION

In March 2020, the World Health Organization (WHO) declared the COVID-19 pandemic, caused by the coronavirus SARS-CoV-2[1,2]. At that moment, several countries initiated measures to contain the spread of the virus, and in Brazil, social isolation devices and support measures for the health system were developed. On March 23, the government of the state of São Paulo declared a state of public calamity[3].

Health authorities used diverse methods of epidemiological surveillance to control the spread of the disease. In addition to social isolation, extreme quarantine measures (i.e., lockdown) were adopted, as well as contact tracing, social distancing, and hygiene measures[2].

Some health institutions implemented telemedicine as a form of remote care to help maintain social distancing[1,2,4]. The digital evolution in recent years has spawned health-related technologies, making telemedicine possible[5,2].

The Center for Innovation and Management in Telemedicine and Telehealth (*Núcleo de Inovação e Gestão em Telemedicina e Telessaúde* – NIGT) was created in a large health institution aimed at facing the COVID-19 pandemic. On April 2, 2020, the center started its activities offering medical advice to patients with flulike illness who were suspected of contracting COVID-19, thus avoiding unnecessary visits to the emergency department, as instructed by the WHO and the Brazilian Ministry of Health at the time, to maintain proper social distancing[1].

The objective of this study was to evaluate the effectiveness of the telemedicine care model implemented to treat and guide patients with flulike illness suspected of COVID-19 during the pandemic.

## METHODS

This study was conducted between April 2 and October 15, 2020. A retrospective study design was used, and data were collected from patients' care standardized electronic health records.

The service was conducted after the patients or the legal guardians signed free and informed consent.

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Telemedicine was implemented in the following forms: teleappointment, telemonitoring, medical teletriage, teleguidance, and request for a formative second opinion.

The effectiveness of this model was evaluated using the following variables: total number of appointments, demographic data, numerical increase of monthly appointments, types of treatment, and treatment outcomes.

The progress of the disease in the period was evaluated by comparing the proportions of patients assisted and those referred to the COVID-19 emergency department, checking the possible progress of the disease severity, and evaluating the proportion of hospitalization and death.

The patient's opinion of the teleappointment was assessed using a standardized questionnaire and conducted telephonically. The assisted patients answered questions regarding resolvability, satisfaction, safety, and clarity of care. For resolvability and safety, they could answer yes or no. For satisfaction and clarity, they were presented a score from 1–10, with 8–10 indicating excellent, 5–7 regular, and 1–4 poor.

### Infrastructure

A total of 92 physicians recruited to work in telemedicine received prior training on legislation, ambience, ethics, and regulations and then followed by a standardized treatment or referral procedure according to the severity of symptoms. Medical certificates, requests for tests and examinations, and prescriptions were sent by mail or picked up in person.

The appointments resulted in four types of referrals:

(i) patients with suspected coronavirus infection but with a mild condition were instructed to remain at home in social isolation;
(ii) patients with severe signs and symptoms were referred to the COVID-19 emergency department;
(iii) patients with other clinical complaints were referred to related specialties for face-to-face appointments; and
(iv) patients who needed emergency care but were not compatible or suspected of COVID-19 were referred to the general emergency department (adult and child).

Referrals followed a standardized protocol according to risk stratification, for referral to face-to-face evaluation (Table 1).

Table 1. Risk stratification.

| Signs and symptoms of severity that determine the need for face-to-face medical evaluation. |
|---|
| 1. Dyspnea (shortness of breath, movement of the nose wings, $O_2$<95% saturation, signs of cyanosis); |
| 2. Persistent fever (does not subside with medications, lasts more than 24 hours, gets higher and higher); |
| 3. Symptoms do not improve over the days; |
| 4. General condition progressively worsening; |
| 5. Occurrence of new symptoms; |
| 6. Changes in the consciousness level, irritability, and mental confusion. |

Institutional COVID-19 Crisis Committee.

### Statistical analysis

Demographic data were described by age and sex. Ages were described as mean, standard deviation, and 95% confidence interval and divided by age groups such as young (0–19 years), adult (20–59), and elderly (60 years and above).

The normality of women and men age distribution was assessed using the Kolmogorov–Smirnov test. The age distribution was not normal (p<0.001) and was compared with Mann-Whitney U test. The proportion of patients referred to the COVID-19 emergency department (and not referred) was compared using a chi-square test. The programs used for statistical analysis were Microsoft Excel 2007 (Microsoft Corp., Redmond, WA, USA) and IBM® SPSS® Modeler version 18.0.

## RESULTS

### Total number of appointments

A total of 3,660 patients were assisted during the study period (Figure 1).

### Demographic data

A total of 2,306 women were telemedicine assisted, of which 1,274 (55%) were adults, 947 (41%) elderly, and 85 (4%) young. Of the 1,354 men assisted, 651 (48%) were adults, 623 (46%) elderly, and 80 (6%) young. The women-to-men ratio was 1.7.

The mean age of the patients assisted was 55.6±17.56 years (95%CI 21–90). The mean age was 55.35±17.10 years (95%CI 21–89) for women and 56.22±18.71 years (95%CI 22–90) for men (Mann-Whitney U test, p=0.10).

### Increase in the number of appointments

Teleappointments started in April 2020. There was a linear growth in the number of appointments until May and mid-June. Between the end of June and July, the number reached a plateau (Figure 1). The peak occurred in the month of July. Afterward, the number began to progressively decline.

There was no difference in referral to the COVID-19 emergency department between the months surveyed ($\chi^2$=3,818; p=0.70).

### Types of treatment

This service provided certificates (8), requests for tests and examinations (183), and prescriptions (7) for treatments at home.

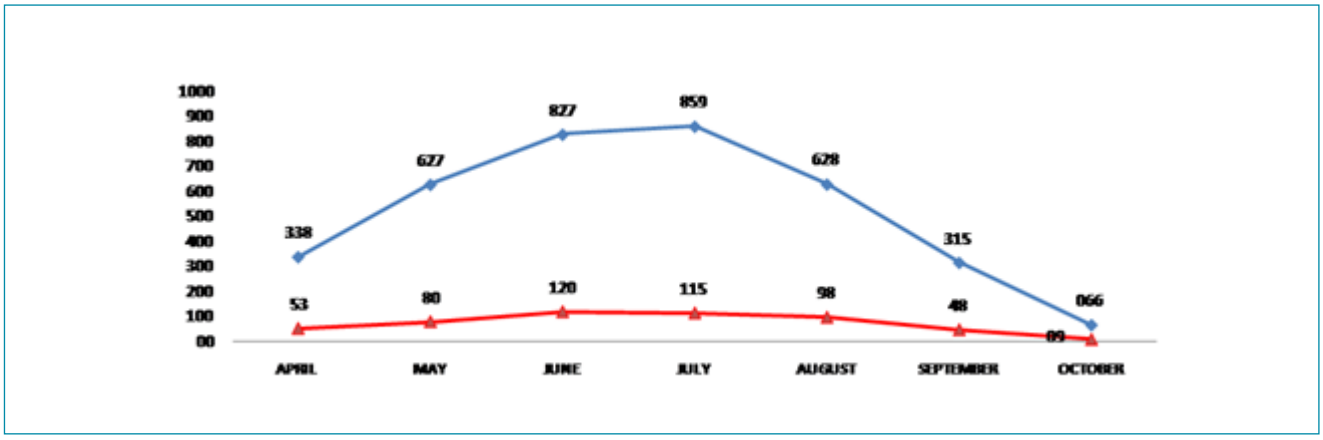


Figure 1. Number of patients attended by month of study (blue). Number of patients referred to the COVID-19 department (red). Number of patients assisted by telemedicine and referred to PS Covid.

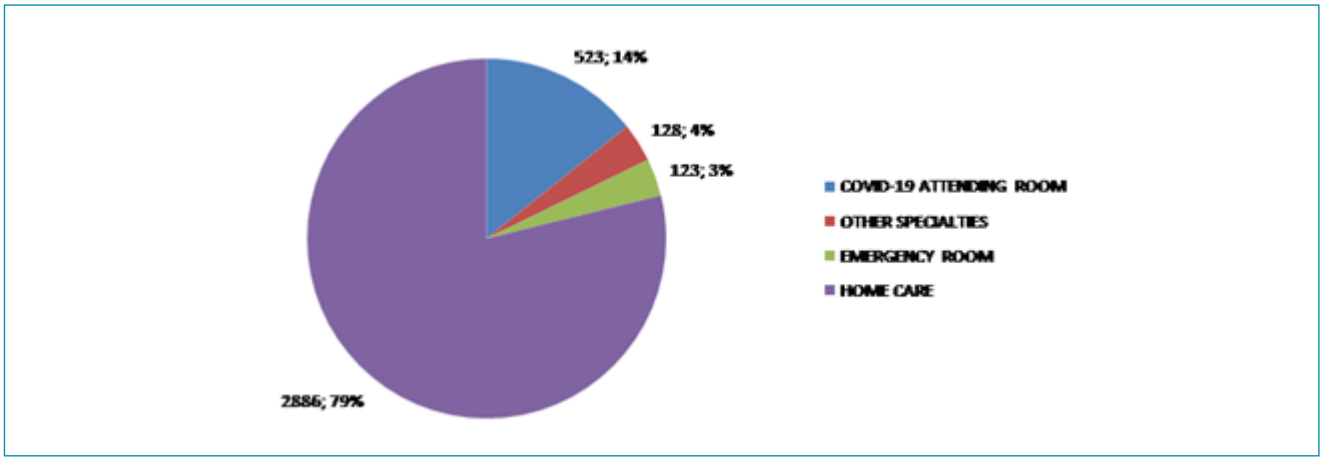


Figure 2. Number and percentage of attending patients referred to the specific departments or specialties. Outcomes.

## Service outcomes

A total of 3,660 patients were assisted. Of these, 523 (14.3%) were referred to the COVID-19 emergency department; 128 (3.5%) were referred to other specialties; 123 (3.4%) were referred to the general emergency department; and 2,886 (78.9%) were monitored via home care. A total of 81 (2.2%) patients were admitted and 13 (0.35%) died (Figure 2).

Regarding death, 8 (62%) were women and 5 (38%) were men. The mean age of those who died was 74.15±13.7 years.

## Evaluation of the service

A total of 2,089 (57%) patients agreed to participate in the survey.

Satisfaction: 95% of patients scored 8–10 (excellent), 5% scored 5–7 (regular), and none scored 1–4 (poor).

Resolvability: 91% (1,905) reported having their problems solved, and 9% (184) said their problem had not been solved.

Safety: 88% (1,846) felt safe, and 12% (243) did not.

Clarity: 97% considered it excellent and 3% regular.

# DISCUSSION

The WHO recommended social isolation to reduce the speed of contagion and protect the health system against overcrowding and avoiding bankruptcy as the main concerns in the fight against the COVID-19 pandemic.

On March 22, 2020, quarantine was decreed in the state of São Paulo as a measure to fight COVID-19[3] and was extended until June 2021. On September 19, the contingency plan was relaxed (orange phase). At the end of September 2020, the plan went into the green phase (less strict), in line with the results found for the peak and decline. There were at least 11 decrees extending the quarantine until October 2020, the end of this study.

Telemedicine has become an appropriate modality to meet these circumstances. Although telemedicine was already

regulated in several countries around the world, in Brazil, it was limited to teleconsulting and teleinterconsulting[6]. Due to the pandemic, this service was authorized as a full-on emergency, while the crisis caused by the coronavirus lasts[7].

The results of this study showed the behavior of patients with suspected COVID-19 who needed guidance and chose virtual appointments. Almost all patients were elderly and adults. A total of 43% of the patients of the studied institution were elderly, a proportion similar to that found in the appointments (42%), and the mean age of deaths corresponded to this age group.

A total of 79% of the patients treated were kept in home care, 4% had other health problems and were referred to other specialties, and 14% had moderate or severe symptoms and were referred to face-to-face appointments. Moreover, 2% of the patients were hospitalized and 0.35% died.

Another study showed similar data as 85% of patients with COVID-19 disease had mild symptoms, 15% had more severe clinical cases, and 5% required intensive care[8].

The mortality of COVID-19 varied according to time of the pandemic and the location studied. In mid-September, the Information Center at the Johns Hopkins University indicated a mortality rate of 3% for Brazil, 10.6% for Mexico, and 9.2% for Ecuador. In India, at that time, it was at 1.6%, only behind the United States[9].

Mortality due to COVID-19 in the state of São Paulo during the study period (April 2 to October 15, 2020) was 0.9/1,000. According to the Civil Registry of Deaths portal, in the state of São Paulo, in this period, there were 40,962 deaths for an estimated population of 44,840,384 inhabitants (0.9/1,000)[10].

In the present study, lethality (mortality due to disease) was evaluated with a result obtained from 3.5/1,000 (13/3,660 patients)[11]. The study by Martinez-Garcia, from March 17 to April 17, 2020, in Spain, found a lethality rate of 6.4/1,000 (2/313 patients)[12].

Telemedicine also served to assist patients with other comorbidities who were referred to other treatments[12]. Most patients considered the service good or excellent. One of the most important health systems in the United States, located in California, showed a similar result[13]. In Brazil, in the city of São Paulo, an emergency service identified that more than 50% of the patients considered the digital triage service acceptable, 90% felt safe about the physician's conduct, and 72% considered their complaints fully solved[4].

## CONCLUSION

Telemedicine provided useful tools for the care, treatment, and monitoring of patients with suspected or confirmed COVID-19 during the pandemic. The patients showed a high level of satisfaction with this type of service.

## AUTHORS' CONTRIBUTIONS

**MAS:** Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. **RVB:** Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Supervision, Writing – review & editing. **ACF:** Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Validation, Visualization, Writing – review & editing. **AHV:** Conceptualization, Data curation, Software. **CV:** Conceptualization, Formal analysis, Writing – original draft, Writing – review & editing.

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# Lung age and respiratory muscle strength in female volleyball players

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## SUMMARY

**OBJECTIVE:** Lung age estimation is a useful approach to determine pulmonary pathologies. In literature, no studies have evaluated and compared lung age in athletes with healthy volunteers. This study aims to compare lung age and respiratory muscle strength in female volleyball players and age-matched healthy volunteers.

**METHODS:** A total of 18 female volleyball players (22.39±4.97 years) and 20 female healthy volunteers (24.85±3.33 years) were included. Pulmonary functions and respiratory muscle strength were assessed using a spirometer and mouth pressure device, respectively. The lung age was calculated using reference equations associated with gender, height, and forced expiratory volume in 1 second.

**RESULTS:** Lung age was significantly lower, and forced expiratory volume in 1 L, forced vital capacity, and maximal inspiratory and expiratory pressure ($cmH_2O$, %) were higher in female volleyball players compared with healthy volunteers ($p \leq 0.05$).

**CONCLUSION:** The lung age and respiratory muscle strength of female volleyball players were better than healthy volunteers. Regular training in female volleyball players may improve respiratory functions and lung age.

KEYWORDS: Lung. Aging. Respiratory muscles. Athletes.

## INTRODUCTION

Lung age estimation is one of the approaches to better understand pulmonary function abnormalities[1]. After the first lung age equation was produced by Morris et al. in 1985[1], many equation varieties have been developed based on age, race, gender, and lung volumes such as forced expiratory volume in 1 second ($FEV_1$)[2]. Most studies have used lung age estimation for motivational smoking cessation counseling[2,3]. The lung age estimation was also used to estimate postoperative pulmonary complication risk in patients with lung cancer[4]. One study focused on patients with morbid obesity who have higher lung age compared with controls[5].

Increased respiratory muscle strength may result in better performance. Regular evaluation of pulmonary functions and respiratory muscle strength is helpful for maintaining performance in athletes[6]. Respiratory symptoms such as asthma-like symptoms, exercise-induced bronchospasm, and cough are commonly reported in athletes[7]. Lung age is a useful method in detecting respiratory pathologies[4]. Furthermore, pulmonary aging is associated with internal factors such as genetic structure and mutations, systemic changes and inflammations, pulmonary structural changes, and lifestyle and external factors such as environmental exposure. Therefore, evaluation of lung age may provide important information about these factors[8]. In the literature, no studies have evaluated and compared lung age in athletes with healthy volunteers. This study aimed to compare lung age and respiratory muscle strength in female volleyball players and age-matched female healthy volunteers. In this study, we tested the hypothesis that female volleyball players may have later pulmonary aging and better respiratory muscle strength compared to female healthy volunteers.

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## METHODS

This was a cross-sectional, retrospective study, in which 18 female volleyball players (22.39±4.97 years) and 20 female healthy volunteers (24.85±3.33 years) who do not follow a regular exercise were included from recorded data during usual care. Participants aged <18 years and who have a history of smoking, COVID-19, and chronic pulmonary disease were excluded. The study was approved by the Ethics Committee of the Gazi University (No. 2021-659).

Demographic characteristics, such as age, weight, height, and body mass index of participants, were regularly recorded during a routine control.

Inspiratory and expiratory muscle strength was measured using a portable mouth pressure device (Micro Medical MicroRM, UK) according to the recommendations from guidelines[9]. The assessments of maximal inspiratory pressure (MIP) and maximal expiratory pressure (MEP) were performed at least five times, and the highest value was selected for analysis. The percentages of inspiratory and expiratory muscle strength predicted values were calculated in accordance with reference equations[10].

$FEV_1$, forced vital capacity (FVC), and $FEV_1$/FVC ratio were evaluated using a portable spirometer (Cosmed, Class II/Internally Powered Equipment, Italy). The highest value of the measured lung volumes at least three times was selected for analysis. The percentages of the predicted values were calculated based on reference equations[11].

The lung age estimation was calculated based on a reference equation for female adults developed by Newbury et al. The formula: Lung age (years)=1.33×Height (cm)−31.98×Observed$FEV_1$−74.65 was used to calculate the lung age of all participants[2].

### Statistical analysis

Statistical analyses were performed using SPSS statistical analysis program version 20.0 (SPSS Inc., Chicago, IL, USA). Descriptive variables were expressed as mean difference, 95% confidence interval (95%CI), means (X)–standard deviation (SD), median–interquartile range (IQR), and percentage (%). Shapiro-Wilk test was performed to evaluate the normal distribution of data. Student's t-test, Mann-Whitney U test, and $\chi^2$ test were used to compare normally distributed, non-normally distributed, and categorical variables, respectively. The level of statistical significance was described as $p \leq 0.05$. In this study, we planned at least 15 participants in each group based on the results of this study using the lung age estimation values for 95% power (G*Power 3.0.10 system, Franz Faul, Universität Kiel, Germany)[12].

## RESULTS

Notably, 18 of 52 players and 20 of 27 healthy volunteers were selected for analysis. As shown in Table 1, demographic characteristics such as age and body mass index were similar in both players and healthy volunteers ($p>0.05$). As shown in Table 2, lung age was significantly lower, and $FEV_1$ (L), FVC (L), MIP ($cmH_2O$, %), and MEP ($cmH_2O$, %) were higher in players compared with healthy volunteers ($p \leq 0.05$). Both MIP and MEP values were below 80% of the predicted values in 3 (16.7%) and 14 (77.8%) players and 9 (45%) and 18 (90%) healthy volunteers, respectively.

## DISCUSSION

This is the first study to evaluate and compare lung age in volleyball players with healthy volunteers. This study shows that the lung age was much close to chronological age in players. Inspiratory and expiratory muscle strength in players was better than healthy volunteers without any chronic disease and regular exercise habits.

Lung age provides important practical information to individuals about their pulmonary functions[13]. Although the exact mechanism of lung aging is not known, structural changes,

Table 1. Demographic and clinical characteristics in volleyball players and healthy volunteers.

| | Volleyball players (n=18) X±SD/median (IQR) | Healthy volunteers (n=20) X±SD/median (IQR) | p-value |
|---|---|---|---|
| Age, years | 22.39±4.97 | 24.85±3.33 | 0.079 |
| Weight, kg | 67.80 (61.45–71.68) | 58.50 (53.25–65.00) | **0.009*** |
| Height, cm | 176.50 (171.75–183.75) | 165.00 (162.75–168.75) | **<0.001*** |
| Body mass index, kg/m² | 21.52±1.64 | 22.01±2.91 | 0.539 |
| Sports age, years | 12.06±5.96 | | |

SD: standard deviation; IQR: interquartile range. Descriptive analyses were expressed as X±SD and median (IQR) for normally and non-normally distributed, respectively. *Mann–Whitney U test ($p<0.05$). Statistically significant p-value were written in bold.

genetic predisposition, environmental exposure, inflammation, and chronic diseases have serious effects on pulmonary aging[8]. A lung age greater than the chronological age indicates poorer pulmonary functions[13]. Many studies used it as a motivation tool for counseling of smoking cessation since smoking causes an increased lung age associated with a decrease in $FEV_1$[1-3,14]. In addition, some studies showed that lung age is a significant predictor in determining postoperative complications and survival[4,13]. Moreover, one study found that lung age is higher in morbidly obese women compared with the control group and is positively correlated with body mass index[5]. The previously mentioned studies have developed different formulas for different ethnicities, ages, and spirometric measurements. However, there is no clear consensus on the most appropriate formula[15,16]. In our study, whether sport improves lung age using the formula developed by Newbury et al.[2] remains the main focus. The current study found that the difference between lung age and chronological age is less in players compared with healthy volunteers. Furthermore, although all participants in groups were nonsmokers, did not have any chronic diseases, and had the same age and body mass index, the lung age of players was better than healthy volunteers. Sports enhance lung growth and pulmonary functions[17]. Therefore, this improvement in players may be attributable to the benefits of physical fitness and steady training.

Previous studies have emphasized better lung function in all athletes compared with nonathletes[18,19]. Contrary to these studies, Mazic et al.[20] found that FVC values of volleyball players were lower than controls and the percentages of $FEV_1$ values were similar in both the groups. Similar to the results of Mazic et al., the results of our study showed that the percentages of lung volumes were not statistically different in both the groups. In addition, the types of sport, age, height, and weight are associated with pulmonary functions[11,20]. Regular physical exercise improves cardiorespiratory fitness and decreases negative consequences of aging[21]. Better lung age of players may be related to the effects of regular training, and sports may delay lung aging. Other factors affecting pulmonary functions in athletes should be investigated. Respiratory events such as exercise-induced bronchoconstriction and dyspnea were the prevalent reasons for decreased performance in athletes[7]. In addition, lung aging is related to the loss of alveolar tissue, decreased elastic recoil of the lung, and chest wall compliance[22]. The use of the lung age can be an option in determining respiratory problems in athletes.

Exercise training recovers cardiorespiratory fitness and improves muscle performance[23]. In the current study, inspiratory and expiratory muscle weakening was common in healthy volunteers who do not regularly exercise compared with players. Regular training may improve respiratory muscle performance in players involved in our study.

Ohya et al.[24] evaluated respiratory muscle strength in different sports branches and showed that the type of sports has

Table 2. Comparison of lung age, respiratory muscle strength, and pulmonary functions in volleyball players and healthy volunteers.

| | Volleyball players (n=18) X±SD/median (IQR) | Healthy volunteers (n=20) X±SD/median (IQR) | Means difference 95%CI/U | p-value |
|---|---|---|---|---|
| Lung age (years) | 27.93±14.02 | 43.01±7.23 | -15.08 (-22.86– -7.29) | **0.001*** |
| Pulmonary function test (L) | | | | |
| FEV1 | 4.28 (3.63–4.65) | 3.15 (3.02–3.39) | 26 | **<0.001**** |
| FVC | 4.37 (4.13–4.76) | 3.72 (3.59–3.98) | 82.5 | **0.004**** |
| Pulmonary function test (%) | | | | |
| FEV1 | 97.50±8.02 | 94.81±5.97 | 2.69 (-1.93–7.31) | 0.245 |
| FVC | 98.22±8.75 | 97.00±7.66 | 1.23 (-4.17–6.63) | 0.648 |
| FEV1/FVC | 98.83±6.25 | 98.31±5.39 | 0.52 (-3.31–4.35) | 0.784 |
| Respiratory muscle strength | | | | |
| MIP ($cmH_2O$) | 89.00±19.44 | 74.15±23.05 | 14.85 (0.73–28.97) | **0.040*** |
| MIP (%) | 95.94±20.04 | 81.20±25.15 | 14.74 (-0.34–29.82) | **0.055*** |
| MEP ($cmH_2O$) | 110.11±21.30 | 90.50±23.14 | 19.61 (4.93–34.30) | **0.010*** |
| MEP (%) | 69.59±13.25 | 57.69±14.61 | 11.90 (2.69–21.12) | **0.013*** |

FEV1: forced expiratory volume in 1 s; FVC: forced vital capacity; MIP: maximal inspiratory pressure; MEP: maximal expiratory pressure; CI: confidence interval; SD: standard deviation. Descriptive analyses were expressed as X±SD and median (IQR) for normally and non-normally distributed, respectively. *Student's t test ($p<0.05$). **Mann-Whitney U test ($p<0.05$). Statistically significant p-value were written in bold.

an impact on MIP in female athletes. Furthermore, the aforementioned study demonstrated that inspiratory muscle strength (74.1 $cmH_2O$) in volleyball players is lower than other players. Inspiratory muscle strength is higher in athletes playing water sports, as previously reported[20,24]. In our study, inspiratory muscle strength (89 $cmH_2O$) was higher than that expected in volleyball players and lower than swimmers, as compared with the study by Ohya et al[24]. Predictably, the respiratory muscles and the diaphragm of the swimmers develop more under pressure in water during breathing. Therefore, swimmers have greater lung volumes and inspiratory pressures compared with volleyball players[20]. The inspiratory muscle training substantially improves sports performance by reducing respiratory and limb muscle fatigue, respiratory workload, and attenuating respiratory muscle metaboreflex[25,26]. The effect of inspiratory muscle training on performance in different sports is still unclear. Future studies should be focused on the effect of inspiratory muscle training on performance in different sports.

The limitation of the study is that height and weight of volleyball players due to sportive characteristics were not similar to healthy volunteers.

In conclusion, lung age and respiratory muscle strength in volleyball players were better than healthy volunteers. Sports and steady training may develop these parameters in players. Further studies are needed to investigate the causes affecting lung age in athletes.

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## AUTHORS' CONTRIBUTIONS

**ZÇ:** Conceptualization, Methodology, Data curation, Writing – original draft, Visualization, Investigation. **NAG:** Conceptualization, Methodology, Visualization, Investigation, Supervision, Writing – review & editing. **FY:** Data curation, Visualization, Investigation, Supervision, Writing – review & editing. **NK:** Data curation, Visualization, Investigation, Supervision, Writing – review & editing.

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# Can serum 8-hydroxy-2'-deoxyguanosine levels reflect the severity of pulmonary arterial hypertension?

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## SUMMARY

**OBJECTIVE:** Oxidative stress plays a pivotal role in the pathogenesis of pulmonary arterial hypertension. 8-Hydroxy-2'-deoxyguanosine is a sensitive biomarker that reflects the degree of oxidative damage to DNA. We investigated whether serum 8-Hydroxy-2'-deoxyguanosine is a clinically useful biomarker for the severity of pulmonary arterial hypertension.

**METHODS:** We measured serum 8-Hydroxy-2'-deoxyguanosine levels in 25 patients (age 37±13 years, 68% women) diagnosed with idiopathic pulmonary arterial hypertension, familial pulmonary arterial hypertension, or pulmonary arterial hypertension associated with congenital heart disease. The severity of pulmonary arterial hypertension was evaluated by six-min walking distance, World Health Organization functional class, and serum brain natriuretic peptide levels. Age and gender-matched 22 healthy subjects served as the control group.

**RESULTS:** The comparison of 8-Hydroxy-2'-deoxyguanosine levels between patients and controls was not statistically different [(19.86±9.79) *versus* (18.80±3.94) ng/mL, p=0.622)]. However, there was a significant negative correlation between 8-Hydroxy-2'-deoxyguanosine levels and six-min walking distance (r= -0.614, p=0.001). Additionally, serum 8-Hydroxy-2'-deoxyguanosine levels in patients with functional class III–IV were significantly higher than those with functional class I–II (functional class III–IV 32.31±10.63 ng/mL *versus* functional class I–II 16.74±6.81 ng/mL, respectively, p=0.003).

**CONCLUSION:** The 8-Hydroxy-2'-deoxyguanosine levels were significantly correlated with exercise capacity (six-min walking distance) and symptomatic status (functional class), both of which show the severity of pulmonary arterial hypertension in patients.

KEYWORDS: 8-Hydroxy-2'-deoxyguanosine. Pulmonary arterial hypertension. Oxidative stress.

## INTRODUCTION

Pulmonary arterial hypertension (PAH) is a severe clinical condition characterized by progressive obstructive remodeling of the distal pulmonary arteries resulting in a rise of pulmonary artery pressure and pulmonary vascular resistance (PVR) and subsequently leading to right heart failure and premature death unless treated[1].

The pathophysiology of PAH is a very complex process that is still not well understood. Genetic and environmental factors can initiate the pathologic process in pulmonary vascular remodeling. These changes involve pulmonary endothelial dysfunction, maladapted immunity, inflammation, proliferation, and phenotypic alterations of pulmonary vascular cells. Particularly, current evidence indicates that inflammation and increased oxidative status play a major role in mediating vascular remodeling and PAH progression[2]. Increased oxidative stress promotes oxidative damage to cellular components such as proteins, lipids, and DNA. It has been shown that DNA damage is increased in human remodeled pulmonary arteries such as pulmonary artery smooth muscle cells (PA-SMCs) and pulmonary artery endothelial cells (PA-ECs) in explanted lungs of patients with PAH[3]. Furthermore, increased DNA damage and/or impaired DNA repair are considered to be the potential stimulators of the proliferative and apoptosis-resistant phenotype, which is described in vascular remodeling of PAH.

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8-Hydroxy-2'-deoxyguanosine (8-OHdG) is the most commonly found oxidative DNA damage product in humans. This oxidative, modified DNA product has extensively been investigated as a marker of the degree of oxidative damage to DNA due to its stability. The increased levels of 8-OHdG are associated with carcinogenesis, cardiovascular disease, and diabetes[4].

To the best of our knowledge, there are no data regarding the DNA damage marker and its relation with the severity of patients with PAH. Thus, we aimed to evaluate the serum 8-OHdG levels and their association with the severity of disease in patients with PAH.

# METHODS

## Study population

Patients aged 18 or older and previously diagnosed with idiopathic PAH (IPAH), familial PAH (FPAH), and PAH associated with congenital heart disease (CHD) by right heart catheterization in our center were included in the study. PAH was defined as mean pulmonary artery pressure over 25 mmHg, pulmonary artery wedge pressure (PAWP) £15 mmHg, and PVR ³3 wood units. Considering the possible increase of oxidative DNA damage in chronic diseases, PAH subgroups associated with chronic diseases (i.e., connective tissue disease, HIV infection, and portal hypertension) and drug-toxin-associated PAH were not included in the study[5-7]. Other exclusion criteria were pulmonary veno-occlusive disease/pulmonary capillary hemangiomatosis, pulmonary hypertension (PH) due to left heart disease, PH due to lung diseases and/or hypoxia, chronic thromboembolic and/or other pulmonary artery obstructions with PH and PH with unclear or multifactorial mechanisms, history of chronic disease, abnormal renal function (creatinine >1.5 mg/dL), abnormal liver functions, acute infections, and neoplasia. Consequently, 25 patients who met the inclusion criteria were enrolled in the study. Age and gender-matched 22 healthy individuals served as the control group who had no medical history of any chronic disease and no abnormal findings in blood tests and transthoracic echocardiography. Patients were classified into three groups according to the WHO functional classification (FC) criteria as follows: FC I–II, FC III, and FC IV. A six-min walk distance (6MWD) was performed according to the standard protocol[8]. Ethical approval was obtained from the local Ethics Committee (N° 2018/333). Informed written consent was provided from all subjects.

## Echocardiographic assessment

Two-dimensional, M-mode, tissue Doppler imaging (TDI), spectral, and color flow Doppler echocardiographic assessments were performed using Vivid E9, 2–4 MHz phased-array transducer (General Electric, USA) in all patients and control subjects. Chambers' quantification and function were evaluated according to the recommendation of the American Society of Echocardiography and the European Association of Cardiovascular Imaging Guidelines. Right ventricular (RV) function was evaluated using tricuspid annular plane systolic excursion (TAPSE), TDI-derived tricuspid lateral annular systolic velocity wave (RV-S'), and RV fractional area changing (FAC) from RV-focused apical four-chamber view. The estimated RV systolic pressure was calculated using the maximum tricuspid regurgitation jet and the estimation of RA pressure based on inferior vena cava size and collapsibility. Left ventricular ejection fraction (LVEF) was calculated using biplane modified Simpson's rule[9].

## Blood sampling for 8-Hydroxy-2'-deoxyguanosine measurement

Blood samples were obtained from a peripheral vein after an overnight fasting and 10-min rest period for the measurements of 8-OHdG, brain natriuretic peptide (BNP), and other basic biochemical variables. Samples were kept frozen at -80°C if not analyzed immediately. Serum 8-OHdG measurement was made using the Elx 800 instrument (BioTek Instruments, Winooski, VT, USA) with a commercial competitive enzyme-linked immunosorbent assay (ELISA) kit (Northwest, NWLSS 8-OHdG ELISA High Sensitivity Kit, Vancouver, Canada), which expressed as ng/ml. The plasma levels of 8-OHdG, echocardiographic variables, and basic characteristics were compared between the groups. The correlation between the plasma levels of 8-OHdG and 6MWD, BNP, and echocardiographic measurements were investigated among patients. Also, 8-OHdG levels were compared across patients' groups classified according to FC.

## Statistical evaluation

SPSS version 20.0 software was used for the statistical analysis. The Student's *t*-test was used for the comparison of parametric qualitative variables between the PAH and control groups. Pearson's correlation analysis was used to assess correlations between continuous variables with normal distribution, and Spearman's rank test was used for the variables with skewed distribution. A $p<0.05$ (two-sided) was considered statistically significant.

# RESULTS

Baseline characteristics and echocardiographic measurements of the patient group (n=25) are presented in Table 1. The mean age of the patients was 37±13 years, and 68% of them were females.

Table 1. Characteristics of patients (n=25).

| Demographics | |
|---|---|
| Age (year) | 37±13 |
| Sex (female/male) | 17/8 |
| BMI (kg/m$^2$) | 24.3±4.7 |
| Diagnosis [n (%)] | |
| IPAH | 5 (20) |
| FPAH | 1 (4) |
| PAH associated with CHD | 19 (76) |
| Clinical Findings | |
| 6MWD (m) | 371.0±146.6 |
| WHO Functional Class [n (%)] | |
| FC I–II | 20 (80) |
| FC III–IV | 5 (20) |
| Clinical signs of right heart failure [n (%)] | 6 (24) |
| Syncope [n (%)] | 1 (4) |
| Heart rhythm [n (%)] | |
| SR | 20 (80) |
| AF | 5 (20) |
| Laboratory Findings | |
| Hemoglobin (g/dL) | 14.1±2.9 |
| Leukocyte (10$^3$/mL) | 7.431±2.026 |
| Platelet (10$^3$/mL) | 230±61 |
| Creatinine (mg/dL) | 0.72±0.22 |
| AST (U/L) | 24±7 |
| ALT (U/L) | 15±8 |
| Uric acid (mg/dL) | 5.7±2.2 |
| Medications [n (%)] | |
| ERA<br>Bosentan/Macitentan/Ambrisentan | 25 (100)<br>5 (20)/19 (76)/1 (4) |
| PDEI<br>Sildenafil/Tadalafil | 18 (72)<br>7 (28)/11 (44) |
| Prostacyclin analogs | 2 (8) |
| Diuretic | 9 (36) |
| Calcium channel blocker | 4 (16) |
| Beta-blocker | 6 (24) |
| Digoxin | 3 (12) |
| Warfarin or DOAC | 4 (16) |

BMI: body mass index; PAH: pulmonary arterial hypertension; IPAH: idiopathic PAH; FPAH: Familial PAH; CHD: congenital heart disease; 6MWD: six-min walking distance; FC: functional class; SR: sinus rhythm; AF: atrial fibrillation; AST: aspartate transaminase; ALT: alanine transaminase; ERA: endothelin receptor antagonist; PDEI: phosphodiesterase inhibitor; DOAC: direct oral anticoagulant.

Almost 80% were PAH associated with CHD. Meanwhile, most of them were in FC I or II, and only 24% had symptoms of right heart failure.

Table 2 shows the comparison of the groups. The comparison of 8-OHdG levels between patients and controls was not statistically different [(19.86±9.79) *versus* (18.80±3.94) ng/mL, respectively, p=0.622)]. Moreover, the comparison of 8-OHdG levels between patients' subgroups (IPAH/FPAH and PAH associated with CHD) was also not statistically different [(14.72±4.81) *versus* (21.48±10.48) ng/mL, respectively, p=0.144)]. However, the levels of 8-OHdG levels were negatively correlated with 6MWD (r=-0.614, p=0.001) (Table 3). Furthermore, the serum 8-OHdG levels in patients with FC III–IV were significantly higher than those with FC I–II (FC III–IV 32.31±10.63 ng/mL *versus* FC I–II 16.74±6.81 ng/mL, respectively, p=0.003) (Figure 1).

The BNP levels were significantly higher in patients compared to control (175.75±76.45 *versus* 15.16±1.79 ng/mL; p=0.002) and showed significantly negative correlation with 6MWD (r= -0.506, p=0.010) (Table 3). Also, serum BNP levels in patients with FC III–IV were significantly higher than those with FC I–II (FC III–IV: 628.00±720.41 ng/mL *versus* FC I–II 62.69±89.64 ng/mL, respectively, p=0.042). There was no association between 8-OHdG and BNP levels.

## DISCUSSION

Our results showed that serum 8-OHdG levels, a marker of DNA damage, were increased significantly in parallel to the severity of FC and negatively correlated with 6MWD among patients with PAH. Both FC and 6MWD are used as noninvasive risk tools for disease severity and risk prediction of mortality of patients with PAH in current risk-scoring systems. They are powerful predictors of survival and correlated with the severity of disease at diagnosis and also during follow-up in PAH[10].

DNA damage has been previously shown to be increased in the lungs of patients with PAH, remodeled arteries, PA-SMCs, and PA-ECs[11]. Furthermore, the oxidative stress that is believed to play a crucial role in the development and progression of vascular remodeling in PAH could also promote DNA damage[12]. Although DNA damage and intrinsic mutagen sensitivity have been shown to increase in PAH[3], to the best of our knowledge, there is no study to evaluate the serum DNA damage markers and disease severity in these patient populations so far.

The assessment of the severity of PAH poses special importance because it is used for guiding medical therapy and determining the timing of lung transplantation. Therefore, numerous biomarkers as noninvasive parameters have been extensively investigated[13]. These markers might be classified as markers of

Table 2. Comparison of basal characteristics, serum brain natriuretic peptide and 8-OHdG levels, and echocardiographic parameters between patients and controls.

| | Patients (n=25) | Control (n=22) | p-value |
|---|---|---|---|
| Age (year) | 37.4±13.6 | 35.8±8.3 | 0.614 |
| Gender (female/male) | 17/8 | 14/8 | 0.763 |
| BMI (kg/m$^2$) | 24.3±4.7 | 25.3±3.7 | 0.469 |
| 8-OHdG (ng/mL) | 19.86±9.79 | 18.80±3.94 | 0.622 |
| BNP | 175.75±76.45 | 15.16±1.79 | 0.002 |
| TRV (m/s) | 3.82±1.20 | 1.00±1.07 | <0.001 |
| SPAP (mmHg) | 72.50±7.80 | 9.54±2.37 | <0.001 |
| RV-FAC (%) | 34.52±9.92 | 49.68±7.53 | <0.001 |
| RV annular peak velocity (cm/s) | 12.08±3.49 | 14.18±2.19 | 0.017 |
| TAPSE (mm) | 18.24±3.58 | 23.77±4.29 | <0.001 |
| LV-Eccentricity index | 1.46±0.28 | 0.98±0.08 | <0.001 |
| RV/LV basal diameter ratio | 1.29±0.28 | 0.75±0.08 | <0.001 |
| RA area (cm$^2$) | 26.98±3.57 | 12.00±2.38 | <0.001 |
| PA (mm) | 31.84±6.69 | 21.19±2.31 | <0.001 |
| LVEF (%) | 65.04±5.45 | 65.18±6.44 | 0.936 |

BMI: body mass index; BNP: brain natriuretic peptide; TRV: tricuspid regurgitation max velocity; SPAP: systolic pulmonary artery pressure; RV-FAC: right ventricular-fractional area change; TAPSE: tricuspid annular plane systolic excursion; LV: left ventricle; RA: right atrium; PA: pulmonary artery; LVEF: left ventricular ejection fraction.

Table 3. Correlation between 8-Hydroxy-2'-deoxyguanosine levels and 6-min walking distance, brain natriuretic peptide, and echocardiographic parameters.

| | 8-Hydroxy-2'-deoxyguanosine | |
|---|---|---|
| | r | p-value |
| 6MWD | -0.614 | 0.001 |
| BNP | 0.341 | 0.095 |
| TRV | 0.228 | 0.284 |
| SPAP | 0.292 | 0.166 |
| RV-FAC | -0.051 | 0.810 |
| RV annular peak systolic velocity | -0.047 | 0.824 |
| TAPSE | -0.117 | 0.579 |
| RA area | 0.308 | 0.144 |
| PA | 0.423 | 0.035 |

6MWD: 6-min walking distance; BNP: brain natriuretic peptide; TRV: tricuspid regurgitation max velocity; SPAP: systolic pulmonary artery pressure; RV-FAC: right ventricular-fractional area change; TAPSE: tricuspid annular plane systolic excursion; LV: left ventricle; RA: right atrium; PA: pulmonary artery

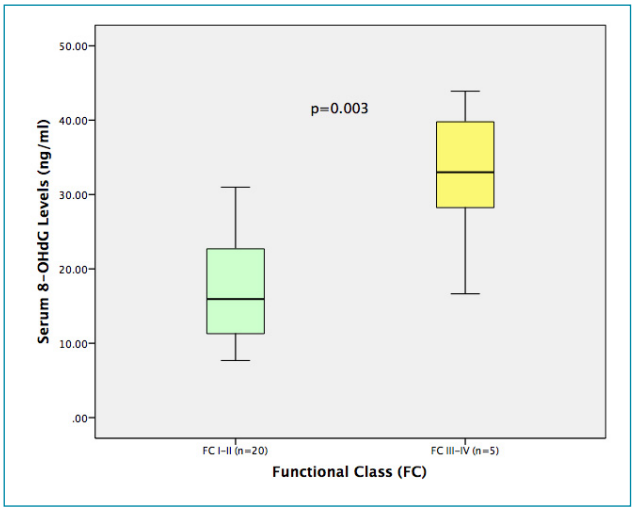


Figure 1. Relationship between serum 8-Hydroxy-2'-deoxyguanosine levels and World Health Organization functional classification among patients with pulmonary arterial hypertension.

vascular dysfunction, inflammation, myocardial stress, and low cardiac output and/or tissue hypoxia. 8-OHdG is a stable biomarker of oxidative damage to DNA that has been shown to be correlated with the severity of several cardiovascular diseases[14,15].

Regarding PAH, only one study from Bowers et al. has shown that plexiform lesions and luminal endothelial cells of concentric intimal fibrosis lesions of explanted lungs of patients with IPAH were intensely stained by 8-OHdG, which is indicating the presence of DNA oxidation[16]. BNP and NT-proBNP, which correlate with myocardial dysfunction, are the most commonly

used validated biomarkers in PAH. In line with our results, these markers also provide information regarding disease severity and prognosis during the diagnosis and follow-up of these patients[17]. Contrary to expectation, there was no correlation between 8-OHdG levels and BNP levels even though they both correlated with the clinical severity of patients in our study. It could be related to different characteristics of these two biomarkers. BNP is mainly secreted from the cardiomyocytes in response to cardiac stretch, and its levels can range widely in short time periods, according to volume status and treatments in patients with heart failure[18]. In contrast, 8-OHdG is a stable marker of oxidative DNA damage, and it seems to reflect an established pathophysiological status[19]; therefore, its levels may not show changes parallel to BNP under acute circumstances.

Some issues and limitations should be pointed out while interpreting our findings. Although on the ground of the knowledge that 8-OHdG is a marker reflecting the degree of oxidative damage to DNA, we could not show any statistical difference regarding the 8-OHdG levels between patients and controls. It could possibly be explained with several reasons. First, a relatively small sample size of the study due to being conducted in a single center may have affected the statistical analysis. Second, 80% of the patients in our study were clinically stable, and their functional class was FC I–II. Studies have shown that there is a negative correlation between patient functional capacity and oxidative status of patients with PAH[13,20]. Third, PAH is associated with CHD, which is comprised of 76% of our study population, even if it shows similar histological features with IPAH, the role of inflammation in its pathogenesis remains controversial[21]. Another worth mentioning point is that the anti-inflammatory effects of PAH-specific treatments are known; however, the evaluation of their effects on oxidative DNA damage and subsequently on 8-OHdG levels could not be possible as there was no treatment naïve patient in our study[22]. Considering these factors mentioned above, the possible low oxidative status of most of our patient population could partly explain the nonsignificant difference of serum 8-OHdG levels between the patients with PAH and the controls. However, it can hardly go beyond speculation.

## CONCLUSION

The serum 8-OHdG level that is an oxidative DNA damage marker significantly correlated with exercise capacity (6MWD) and symptomatic status (FC) of the patients with PAH. However, the abovementioned multiple factors, which may have affected the results of the study, could not be excluded. 8-OHdG might be a potential candidate as a noninvasive parameter for the classification of severity of the patients with PAH; however, it requires further research.

## AUTHORS' CONTRIBUTIONS

**FYC:** Conceptualization, Data curation, Formal analysis, Project administration, Writing – original draft, Writing – review & editing. **ST:** Conceptualization, Data curation, Writing – review & editing. **MK:** Conceptualization, Formal analysis, Writing – original draft, And Writing – review & editing.

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

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## SUMMARY

**OBJECTIVES:** Mean platelet volume is a simple biomarker for inflammatory disease. The purpose of this study is to evaluate the role of mean platelet volume in distinguishing adult-onset Still's disease from sepsis.

**METHODS:** We retrospectively selected 68 patients with adult-onset Still's disease and 55 patients with sepsis between January 2015 and December 2019. Related laboratory data were collected and analyzed.

**RESULTS:** There were no significant differences in white blood cell counts, neutrophils, lymphocytes, and C-reactive protein between adult-onset Still's disease group and sepsis group. However, patients in adult-onset Still's disease group showed higher ferritin and platelets and lower mean platelet volume and platelet distribution width than those in sepsis group ($p<0.01$ for both). Receiver operating characteristic curve analysis was performed to distinguish adult-onset Still's disease and sepsis. The area under the curve of mean platelet volume was 0.761 (95%CI 0.673–0.849), with a sensitivity of 79.1%, a specificity of 63.3%, and a cutoff value of 10.9 fL. In contrast, the area under the curve of combined ferritin and mean platelet volume was 0.90l (95%CI 0.837–0.965), with higher sensitivity (82.8%) and specificity (96.2%). Therefore, mean platelet volume could be used as a supplementary indicator to distinguish adult-onset Still's disease from sepsis.

**CONCLUSION:** We suggest that mean platelet volume could be used as a supplementary biomarker for differential diagnosis of adult-onset Still's disease and sepsis in addition to ferritin.

KEYWORDS: Mean platelet volume. Still's disease, adult-onset. Sepsis.

## INTRODUCTION

Adult-onset Still's disease (AOSD) is a rare condition characterized by leukocytosis, fever, arthralgia, and rash[1]. Due to the lack of specific biomarkers, it is difficult to differentiate AOSD from common diseases, such as malignancy, rheumatic diseases, and infections[1-3]. Sepsis, a dangerous and fatal disease, is difficult to be distinguished from AOSD[4]. Several studies show that signs, ferritin, and interleukin-18 (IL-18) could be used to identify AOSD and sepsis; however, none of them are specific[5]. Therefore, complementary indexes that can distinguish between these two diseases are needed.

Mean platelet volume (MPV) is a traditional biomarker of inflammation that can be measured in routine hematological examination[6,7]. Previous studies demonstrated low level of MPV in patients with rheumatoid arthritis and systemic lupus erythematosus[8,9]; however, MPV in AOSD and sepsis remains unclear. This study aimed to investigate the role of MPV in differential diagnosis of AOSD and sepsis and compare the role of MPV, C-reactive protein (CRP), and ferritin.

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# METHODS

## Subjects

A retrospective study was performed between January 2015 and December 2019. The study participants were patients admitted to the First Affiliated Hospital of Nanjing Medical University. The patients were divided into two groups: AOSD group (n=68) and sepsis group (n=55). Patients who met the Yamaguchi criteria[1] (meeting at least five criteria with two or more major criteria, no exclusion criteria); age ≥18 years; first diagnosed in our hospital without hematological disease, glucocorticoids, and other autoimmune diseases; and received no chemotherapy were included in AOSD group. The major inclusion criteria are (1) fever >39°C for at least 1 week, (2) joint pain or arthritis that lasts 2 weeks or more, (3) typical skin rash, and (4) leukocytosis ≥$10\times10^9$/L with at least 80% granulocytes. The minor inclusion criteria are

(1) sore throat,
(2) splenomegaly/lymphadenopathy,
(3) the absence of rheumatoid factor or antinuclear antibodies, and
(4) impaired liver function. Patients diagnosed with sepsis (meeting the American College of Chest Physicians/Society of Critical Care Medicine Consensus Conference criteria[10]) were assigned in the sepsis group.

Patients were excluded if they have diseases that affect platelet parameters. This study was approved by the Ethics Committee of the local hospital and was in accordance with the guidelines of the Declaration of Helsinki.

# VARIABLES

All variables (i.e., demographics, clinical features, and laboratory values) were obtained from electronic medical records. Complete blood count (before any treatment) was detected using a Sysmex XE 2100 analyzer (Sysmex, Hyogo, Japan), C-reactive protein (before any treatment) was measured by a BN II nephelometer (Dade Behring, Marburg, Germany), and ferritin (before any treatment) was evaluated on a Unicel DXI 800 (Beckman Coulter, Brea, CA, USA).

## Statistical analysis

The parametric quantitative data were expressed as mean and standard deviation and evaluated by chi-square test. Nonparametric quantitative data were displayed as median (interquartile range) and estimated with Wilcoxon test. Qualitative data were represented as number (percentages). The Student's *t* test and Mann-Whitey U test were used to compare the difference between the groups. Spearman's correlation analysis was used to evaluate the correlation between variables. The best cutoff value was confirmed with receiver operating characteristics (ROC) curve analysis. All analyses were conducted with SPSS (SPSS 21 Inc., Chicago, IL, USA). A $p<0.05$ was considered statistically significant.

# RESULTS

## Characteristics

Table 1 summarizes the characteristics of the study patients. The median ages of AOSD group (male-to-female ratio: 23:45) and sepsis group (male-to-female ratio: 27:28) were 40 and 33 years, respectively. The main clinical characteristics and signs of AOSD group and sepsis group are displayed in Table 1.

## Comparison of variables between the two groups

A comparison was made to find the difference between the two independent groups. The levels of ferritin, platelets, MPV, and platelet distribution width (PDW) were found to be 2206.6 (31.2–15000) µg/L, $261.51\times10^9$/L, 10.08 fL, and 11.85% for AOSD group and 404.65 (14.2–1507) µg/L, $163.44\times10^9$/L, 11.14 fL, and 14.00% for sepsis group, respectively, which showed significant difference between the groups ($p<0.001$ for both) (Table 2). Also, the levels of ferritin and platelets were higher and those of MPV and PDW were lower in AOSD group compared to sepsis group. In addition, there was no difference in white blood cell (WBC) count, neutrophils, lymphocytes, and CRP values between the two groups ($p>0.05$ for both) (Table 2).

Table 1. Clinical features of the study patients.

| | Patients with sepsis n=55 | Patients with AOSD n=68 |
|---|---|---|
| Fever, n (%) | 43 (93.5) | 62 (91.2) |
| Arthralgia/arthritis, n (%) | 2 (4.3) | 32 (47.1) |
| Myalgia, n (%) | 10 (21.7) | 21 (30.9) |
| Typical skin rash, n (%) | – | 26 (47.2) |
| Sore throat, n (%) | 3 (6.5) | 31 (45.6) |
| Lymphadenopathy, n (%) | 5 (10.9) | 7 (10.3) |
| Hepatomegaly/splenomegaly, n (%) | 3 (6.5) | 3 (4.4) |

AOSD: adult-onset Still's disease.

## Correlations between MPV and other variables

The correlation between MPV and clinically relevant variables in AOSD group and sepsis group was assessed. The results showed that MPV was positively correlated with PDW (r=0.830, p<0.001) and inversely correlated with WBC count, lymphocytes, neutrophils, platelets, CRP, and ferritin (r=0.060, p=0.524; r=0.158, p=0.090; r=0.047, p=0.619; r=0.509, p<0.001; r=0.003, p=0.976; r=0.076, p=0.473, respectively). However, only the correlation between MPV and platelets or PDW was significant (Table 3).

Table 2. Clinical data of patients with adult-onset Still's disease or sepsis.

| | Patients with sepsis n=55 | Patients with AOSD n=68 | p-value |
|---|---|---|---|
| Age, years | 40 (18–68) | 33 (18–74) | 0.239 |
| Sex, male/female | 27/28 | 23/45 | 0.088 |
| WBC ($\times10^9$/L) | 15.27±8.58 | 15.17±8.63 | 0.948 |
| Lymphocyte ($\times10^9$/L) | 1.17±1.17 | 1.38±0.71 | 0.223 |
| Neutrophil ($\times10^9$/L) | 9.45±7.15 | 13.51±8.12 | 0.870 |
| Platelet ($\times10^9$/L) | 163.44±96.58 | 261.51±118.47 | <0.001 |
| MPV (fL) | 11.14±1.09 | 10.08±1.11 | <0.001 |
| PDW (%) | 14.00±2.93 | 11.85±2.48 | <0.001 |
| CRP (mg/L) | 92.39±73.32 | 99.95±63.51 | 0.553 |
| Ferritin (µg/L) | 404.65 (14.2–1507) | 2206.6 (31.2–15000) | <0.001 |

AOSD: adult-onset Still's disease; WBC: white blood cell; MPV: mean platelet volume; PDW: platelet distribution width; CRP: C-reactive protein.

## Comparison of roles of MPV, CRP, and ferritin in AOSD group and sepsis group

We identified the optimal cutoff value of variables (including CRP, platelets, MPV, PDW, and ferritin using ROC curve) in predicting AOSD and found that the variables were significantly different between AOSD and sepsis groups. The area under the curve (AUC) of ferritin (AUC 0.872, 95%CI 0.814–0.949, sensitivity 73.8%, specificity 90.0%) gave the best result, followed by MPV (AUC 0.761, 95%CI 0.673–0.849, sensitivity 79.1%, specificity 63.3%) (Table 4). Then, the performance of the combined MPV and ferritin was analyzed and gave a better result with the highest AUC (AUC 0.901, 95%CI 0.837–0.965, sensitivity 82.8%, specificity 96.2%), which could be helpful to distinguish AOSD from sepsis (Figure 1).

Table 3. Correlations between Mean platelet volume and variables.

| | Correlation coefficient (r) | p-value |
|---|---|---|
| WBC ($\times10^9$/L) | -0.060 | 0.524 |
| Lymphocytes ($\times10^9$/L) | -0.158 | 0.090 |
| Neutrophils ($\times10^9$/L) | -0.047 | 0.619 |
| RDW (%) | 0.173 | 0.063 |
| Platelets ($\times10^9$/L) | -0.509 | <0.001 |
| PCT | -0.325 | <0.001 |
| PDW | 0.830 | <0.001 |
| CRP (mg/L) | -0.003 | 0.976 |
| Ferritin (µg/L) | -0.076 | 0.473 |

CRP: C-reactive protein; WBC: white blood cell; RDW: red cell distribution width; PCT: plateletcrit; MPV: mean platelet volume; PDW: platelet distribution width.

Table 4. The performance for each tested markers.

| | Cutoff | AUC | 95%CI | Sensitivity | Specificity | p-value |
|---|---|---|---|---|---|---|
| CRP(mg/L) | 84.0 | 0.558 | 0.452–0.664 | 58.5 | 49.0 | 0.282 |
| Ferritin(µg/L) | 1086.4 | 0.872 | 0.814–0.949 | 73.8 | 90.0 | <0.001 |
| PLT(fL) | 190.0 | 0.739 | 0.652–0.827 | 69.1 | 63.6 | <0.001 |
| PCT(mg/L) | 0.25 | 0.711 | 0.615–0.807 | 50.7 | 75.5 | <0.001 |
| MPV(fL) | 10.9 | 0.761 | 0.673–0.849 | 79.1 | 63.3 | <0.001 |
| PDW(fL) | 12.5 | 0.737 | 0.644–0.829 | 65.3 | 70.1 | <0.001 |
| Ferritin+MPV | – | 0.901 | 0.837–0.965 | 82.8 | 96.2 | <0.001 |

CRP: C-reactive protein; PLT: platelet count test; PCT: plateletcrit; MPV: mean platelet volume; PDW: platelet distribution width; AUC: area under the curve.

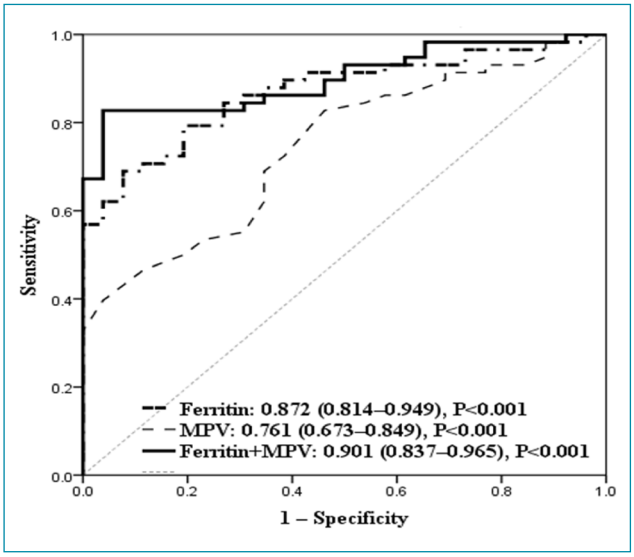


Figure 1. Comparison of AUC between ferritin, MPV, and combined ferritin and MPV.

## DISCUSSION

The clinical manifestations and characteristics of AOSD are nonspecific; therefore, it is difficult to distinguish AOSD from infectious diseases, especially sepsis. AOSD is difficult to cure and easy to relapse, with 9–10% mortality rate when combined with complications such as pneumonia[11,12]. Timely and accurate diagnosis could relieve mental and physical pain in patients. Thus, the sooner AOSD is diagnosed, the better the prognosis will be. Previous literature showed that signs, ferritin, and IL-18 could distinguish AOSD from sepsis; however, none of them were specific.

MPV has been considered a predictive biomarker for sepsis[6,7,13,14]. One study showed that the baseline MPV of patients with culturally- proven sepsis was comparatively higher than that of patients in control group[6]. Furthermore, high cord blood and day-3 MPV can be used as a surrogate marker of predicting early-onset sepsis and associated mortality in preterm neonates[13]. However, few studies addressed the benefit of MPV in patients with AOSD. In this study, we found that MPVs in AOSD group were remarkably lower than those in sepsis group (10.08 fL *versus* 11.14 fL, p=0.001), while ferritin in AOSD group was considerably higher than that in sepsis group (2206.6 [31.2–15000] µg/L *versus* 404.65 [14.2, 1507] µg/L). ROC curve showed ferritin had better performance than MPV in differentiating AOSD from sepsis (AUC 0.872 *versus* 0.761), and the AUC of the combined MPV and ferritin was 0.901. MPV is a sensitive biomarker of platelet morphology that is related to the increased production of platelets, despite its destruction or consumption. Ferritin is a useful marker for diagnosis, disease activity assessment, and prognosis. This means that if a patient (sepsis or AOSD) has high ferritin and low MPV, then the patient is considered primarily to have AOSD or sepsis. MPV in AOSD group was strongly correlated with the levels of platelets and PDW, which had been reported as inflammation biomarkers[15]. Meanwhile, consistent with other reports, we found CRP is the most commonly used reaction protein that is neither different between patients with AOSD and sepsis [15,16] nor correlated with MPV. Therefore, we speculated that serum MPV could represent a complementary marker to ferritin for differential diagnosis between AOSD and sepsis (sensitivity 79.1%, specificity 63.3%) in febrile patients with indistinguishable or similar clinical and laboratory features. The mechanism of elevated MPV in inflammatory conditions remains unclear. Numerous studies demonstrated that MPV increased in patients with sepsis and hypothesized that activated platelets altered in terms of shapes and sizes[17].

MPV was used to describe the average size of platelets in the blood and was routinely measured as part of an automated full blood count request. Given that no objective laboratory results can help physicians discriminate AOSD and sepsis at admission, MPV might be useful for the differential diagnosis of AOSD and sepsis, which is more rapid and more cost-effective than other markers (although the performance of MPV did not surpass that of ferritin).

The research had some limitations due to its retrospective nature. First, limited data of patients were included. Second, no validation group validates the conclusion. Third, we did not validate different MPV measurement methods in individual laboratories. Therefore, a multicenter study with more patients with AOSD is needed.

## CONCLUSIONS

MPV might be a rapid, cost-effective, and helpful marker for the differential diagnosis between AOSD and sepsis in regard to indistinguishable disease patterns.

## AUTHORS' CONTRIBUTIONS

**LL:** Conceptualization, Data curation, Formal analysis, Investigation, Writing – original draft. **LZ:** Conceptualization, Data curation, Formal analysis, Writing – review & editing. **JJ:** Conceptualization, Data curation, Formal analysis, Writing – review & editing. **XD:** Conceptualization, Data curation, Formal analysis, Writing – review & editing. All authors contributed equally to this work.

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# Evaluation of the effect of antibiotics used during parenteral nutrition treatment on Candidemia

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## SUMMARY

**OBJECTIVE:** Parenteral nutrition is an important risk factor for candidemia. In this risk analysis study, the effect of previous antibiotic administration apart from the length of hospital stay, duration of Parenteral nutrition treatment, and *Candida* score parameters on developing candidemia was evaluated in the non-neutropenic patients receiving Parenteral nutrition treatment.

**METHODS:** In this double center, retrospective, and cross-sectional study, the data of patients who received Parenteral nutrition treatment were collected. Patients with or without candidemia after the initiation of Parenteral nutrition treatment were compared in terms of demographic features, *Candida* score, length of hospital stay, duration of Parenteral nutrition treatment, and previous use of antibiotics. Then, predictor factors affecting the probability of candidemia during *Candida* growth time were determined by the Cox regression analysis.

**RESULTS:** A total of 148 patients (59.5% males) were included and 16 (10.81%) of these had candidemia after initiation of parenteral nutrition treatment. The median (min–max) duration of parenteral nutrition treatment was 11 (4–72) days and the *Candida* growth time was 13 (7–29) days. Statistically significant differences were found between patients with or without candidemia groups in terms of length of hospital stay ($p<0.001$), duration of parenteral nutrition treatment ($p<0.001$), and *Candida* score ($p<0.001$). To determine the effect of these variables and antibiotics on candidemia, length of hospital stay [Hazard Ratio 1.030; $p=0.021$] and piperacillin–tazobactam (Hazard Ratio 5.626; $p=0.030$) were found significant and independent risk factors on the development of candidemia.

**CONCLUSION:** There are some well-known risk factors including length of hospital stay, duration of Parenteral nutrition treatment, and *Candida* score; the potential impact of piperacillin–tazobactam administration should also be considered since they may be effective on the development of candidemia.

KEYWORDS: Parenteral nutrition. Candidemia. Risk factors. Antibiotic. Piperacillin-Tazobactam.

## INTRODUCTION

Hospital-acquired *Candida* and bloodstream infections (BSI) represent approximately 9% of all nosocomial BSI[1]. In a multi-centered, point prevalence study conducted on the sepsis cases with causative agents in the intensive care unit (ICU), the rate of *Candida* was determined to be 4.7%[2]. While the candidemia-related mortality rate was 83%, invasive candidiasis was found to be an independent risk factor for mortality[3]. Parenteral nutrition (PN) is consistently identified as an independent risk factor for candidemia in both neutropenic and non-neutropenic patients. The mortality rate of *Candida* catheter-related BSI in patients on PN treatment was found 30%[4]. Potential mechanisms that may be responsible for the increase in the candidemia risk include intestinal mucosal atrophy and subsequent translocation of microorganisms

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or endotoxins, hyperglycemia, nutrient-rich components supporting bacterial and fungal growth, and indwelling parenteral access devices[5]. According to a retrospective, case–control study, both hospital and ICU length of hospital stay (LOS) are time-dependent risk factors for candidemia (p<0.001)[6]. PN exposure time is a risk factor for the development of candidemia, especially in critically ill patients. According to Chow et al., PN duration was a significant risk factor for the development of candidemia in all patients with or without *Candida albicans* (p<0.01)[4]. PN is lifesaving when it is needed but besides the benefit of PN, determining the risk of developing candidemia in patients and initiation of the most appropriate antifungal treatment at the most propitious time can minimize the occurrence of PN-related candidemia[5]. PN treatment indications, safe administration techniques, and duration are reported in both the American Society for Parenteral and Enteral Nutrition (ASPEN) and European Society for Clinical Nutrition and Metabolism (ESPEN) guidelines[7,8]. León et al. developed a bedside "*Candida* score" to decide early antifungal treatment in non-neutropenic critically ill patients with *Candida* colonization. The "Candida score" for a cutoff value of 2.5 points were as follows for deciding early antifungal treatment: PN, surgery, multifocal colonization (1 one point each), and severe sepsis (2 two points)[9].

In this risk analysis study, the effect of antibiotic administration apart from LOS, duration of PN treatment, and *Candida* score (i.e., clinical sepsis, PN administration, surgery, and multifocal colonization) parameters on developing candidemia was evaluated in the non-neutropenic patients receiving PN treatment.

# METHODS

## Patients

In this double-center, retrospective, and cross-sectional study, the data of patients who received PN treatment between January 2019 and December 2019 were collected. Patients aged 18 years or older, who were non-neutropenic (neutropenia: neutrophil count $<0.1\times10^9$ cells/L) and did not receive chemotherapy during PN treatment, who had available culture test results, and who did not have candidemia before PN treatment were included. PN treatment was evaluated by the clinical nutrition team, and only the patients who indicated PN treatment were able to receive the PN treatment. Multi-chamber bag PN was administered to all patients in this study (OliClinomel® N4-550E; Baxter Healthcare Corporation). The patients with and without candidemia after PN were compared in terms of demographic features (i.e., age, gender, and admitted department), LOS, duration of PN treatment, leukocyte and platelet counts, and concomitantly administration of antibiotics.

The time from the start of PN treatment until the development of candidemia was determined as the *Candida* growth time. According to blood culture tests, *Candida* species was reported as *C. albicans* for all study patients by the microbiology laboratory. The patients who were prescribed antibiotics before PN treatment were excluded from this study. Patients who were prescribed at least one antibiotic and only their antibiotic treatments that have been initiated after the initiation of PN treatment and before the development of candidemia were included in this study. The duration of antibiotic treatment is at least 7 days for all study patients. Also, the patients who received fluconazole prophylaxis before the development of candidemia were excluded from this study. This study protocol was approved by the Çukurova University Ethics Committee (Decision No. 2020/56-105).

## Statistical analysis

Chi-square test, Fisher's exact test, Student's *t*-test, Mann–Whitney U test, Poisson regression, and Cox regression analysis, whichever appropriate, were performed. Our collective data met the criteria of a Cox distribution (multivariate model), and the appropriate model had been adjusted to determine the independent predictors of the patient outcome. According to the literature and clinical experience, we created two different models, namely, a model with *Candida* score, LOS, and duration of PN treatment and another model with narrow and broad-spectrum antibiotics administered during PN treatment. For all tests, p<0.05 was considered statistically significant. According to the Omnibus test in Cox regression analysis, having all the independent variables in our example models, we have p-values for first (–2 Log-Likelihood: 51.84; p=0.025) and second models (-2 Log-Likelihood: 38.80; p=0.017), indicating statistically significant overall model. IBM SPSS Statistics 23.0 software was used to analyze and evaluate the data. Since the medical literature does not contain similar studies, the sample size could not be calculated. However, at the end of this study, the power analysis result was determined as 98.06% (G*Power 3.1 Statistical Power Analysis).

# RESULTS

## Patients' characteristics

A total of 148 patients [88 (59.5%) males] with the mean (standard deviation, SD) age of 63.92±18.85 years were included. Half of the patients were admitted in ICU (50.0%) wards. The median (min–max) LOS was 22 days (5–206 days). The most commonly prescribed antibiotics in these patients were cephalosporins (n=60) (Table 1).

Table 1. Distribution of demographic characteristics and antibiotic treatments of the study population (n=148).

| | |
|---|---|
| Gender, male, n (%) | 88 (59.5) |
| Age, mean±SD | 63.92±18.85 |
| Leukocyte, median (min–max) | 10170 (1300–37000) |
| Platelet, median (min–max) | 242000 (18000–821000) |
| Number of patients in ICU, n (%) | 74 (50.0) |
| LOS, median (min–max) days | 22 (5–206) |
| Duration of PN treatment, median (min–max) days | 11 (4–72) |
| *Candida* growth time, median (min–max) days | 13 (7–29) |
| *Candida* score, median (min–max) | 2 (1–5) |
| Candidemia, n (%) | 16 (10.8) |
| Use of antibiotics (narrow spectrum), n (%) | 71 (48.0) |
| Use of antibiotics (broad spectrum), n (%) | 131 (88.5) |
| Antibiotic combinations (2 or more), n (%) | 91 (61.5) |
| Carbapenems | 59 (39.9) |
| Tigecycline | 27 (18.2) |
| Piperacillin–tazobactam | 22 (14.9) |
| Fluoroquinolones | 15 (10.1) |
| Macrolides | 5 (3.4) |
| Cephalosporins | 50 (40.3) |
| Cefazolin | 12 (8.1) |
| Glycopeptides | 20 (13.5) |
| Aminoglycosides | 15 (10.1) |
| Metronidazole | 29 (19.6) |
| Colistin | 8 (5.4) |

SD: standard deviation; ICU: intensive care unit; LOS: length of hospital stay; PN: parenteral nutrition.

It was found that 16 (10.81%) patients had candidemia diagnosis after the initiation of PN treatment. In these patients, the median (min–max) duration of PN treatment was 11 (4–72) days, and the median (min–max) duration of *Candida* growth time after the initiation of PN treatment was 13 (7–29) days. In addition, antibiotic polypharmacy (2 or more) was determined in 91 (61.5%) patients (Table 2).

## Correlation and regression analyses

Statistically significant differences were found between the with and without candidemia groups in terms of LOS ($p<0.001$), duration of PN treatment ($p<0.001$), and *Candida* score ($p<0.001$) (Table 2). Thirty (20.3%) patients were identified as high risk (≥3 points) according to the *Candida* score. There was a significant relationship between the patients with high risk according to the *Candida* score and the candidemia diagnosis ($p<0.001$). In addition, according to the Poisson regression analysis, the *Candida* score was a significant and independent predictor of the candidemia diagnosis ($p<0.001$). For every extra one point in *Candida* score, 1.169 (95%CI 1.110–1.231) times more candidemia was diagnosed (16.9% higher risk). However, the cutoff value (high risk as three or more points) for *Candida* score was not a significant predictor of the candidemia diagnosis ($p=0.224$).

A Cox regression was run to predict whether a patient treating with PN has a diagnosis of candidemia based on the *Candida* score, LOS, duration of PN treatment, narrow and broad-spectrum antibiotics (i.e.., carbapenems, tigecycline, piperacillin–tazobactam, cephalosporins, glycopeptides, and colistin) prescribed after the initiation of PN treatment. Since the number of patients who were prescribed fluoroquinolones, macrolides, cefazolin, aminoglycosides, and metronidazole in the candidemia (+) group was <2, these drugs were not included in the Cox regression analysis to ensure the validity of our model (Table 2). For every extra day in LOS, 1.030 (95%CI 1.004–1.057) times more candidemia risk was determined ($p=0.021$). Also, the hazard ratio (HR) for piperacillin–tazobactam (HR=5.626) indicates that patients who prescribed piperacillin–tazobactam treatment had a higher risk of candidemia than patients who do not ($p=0.030$). In contrast, the duration of PN treatment, *Candida* score, and other antibiotic treatments were not significant risk factors ($p>0.05$) (Table 3).

# DISCUSSION

Candidemia was found in 10.81% of the patients who received PN in this study, which appears higher than the findings of (2–6%) other studies in the literature[10,11]. However, since all the patients who received PN treatment in the hospital were not included, our result is not reflecting the actual incidence due to the design of this study. There were significant differences between groups with and without candidemia in terms of LOS, duration of PN treatment, and *Candida* score. According to the Cox regression analysis, LOS and piperacillin–tazobactam (broad-spectrum antibiotic)

Table 2. Distribution of demographic characteristics and antibiotic treatments by candidemia.

| | Candidemia (+) group (n=16) | Candidemia (-) group (n=132) | p-value |
|---|---|---|---|
| Gender, male, n (%) | 9 (56.2) | 79 (59.8) | 0.78 |
| Age, mean±SD | 62.94±13.92 | 64.04±19.40 | 0.53 |
| Leukocyte, median (min–max) | 10170 (4800–17100) | 10150 (1300–37000) | 0.42 |
| Platelet, median (min–max) | 247500 (113000–821000) | 241000 (18000–734000) | 0.62 |
| LOS, median (min–max) days | 52 (17–206) | 21 (5–127) | <0.001* |
| Duration of PN treatment,median (min–max) days | 21 (9–69) | 10.5 (4–72) | <0.001* |
| *Candida* growth time,median (min–max) days | 13 (7–29) | 0 | – |
| *Candida* score, median (min–max) | 3 (1–5) | 2 (1–4) | <0.001* |
| Use of antibiotics(narrow spectrum), n (%) | 8 (50.0) | 66 (51.5) | 0.24 |
| Use of antibiotics(broad spectrum), n (%) | 16 (100) | 120 (90.9) | 0.21 |
| Antibiotic combinations(2 or more), n (%) | 9 (56.3) | 82 (62.1) | 0.85 |
| Carbapenems | 9 (56.2) | 50 (37.8) | 0.16 |
| Tigecycline | 5 (31.2) | 22 (16.6) | 0.15 |
| Piperacillin–tazobactam | 4 (25.0) | 18 (13.6) | 0.22 |
| Fluoroquinolones | 0 (0) | 15 (11.3) | 0.15 |
| Macrolides | 1 (6.2) | 4 (3.0) | 0.54 |
| Cephalosporins | 4 (25.0) | 56 (42.4) | 0.16 |
| Cefazolin | 0 (0) | 12 (9.0) | 0.09 |
| Glycopeptides | 3 (18.7) | 17 (12.8) | 0.53 |
| Aminoglycosides | 0 (0) | 15 (11.3) | 0.32 |
| Metronidazole | 1 (6.2) | 28 (21.2) | 0.27 |
| Colistin | 2 (12.5) | 6 (4.5) | 0.45 |

*p<0.05. SD: standard deviation; LOS: length of hospital stay; PN: parenteral nutrition.

Table 3. Cox proportional hazard regression analysis.

| | B | SE | Wald | Df | p-value | Hazard ratio(HR) | 95%CI for HR |
|---|---|---|---|---|---|---|---|
| Length of hospital stay* | 0.030 | 0.013 | 5.306 | 1 | 0.021 | 1.030 | 1.004–1.057 |
| Duration of PN treatment | 0.002 | 0.082 | 8.171 | 1 | 0.364 | 1.002 | 0.998–1.006 |
| *Candida* score | -0.975 | 0.639 | 2.332 | 1 | 0.127 | 0.377 | 0.108–1.318 |
| Carbapenems | 1.214 | 0.902 | 1.812 | 1 | 0.178 | 3.366 | 0.575–9.706 |
| Tigecycline | 1.414 | 0.903 | 2.453 | 1 | 0.117 | 4.112 | 0.701–14.123 |
| Piperacillin–tazobactam* | 1.727 | 0.795 | 4.724 | 1 | 0.030 | 5.626 | 1.185–16.712 |
| Cephalosporins | -0.180 | 1.264 | 0.020 | 1 | 0.887 | 0.835 | 0.070–9.941 |
| Glycopeptides | 1.775 | 1.034 | 2.946 | 1 | 0.086 | 5.902 | 0.777–14.800 |
| Colistin | -0.186 | 1.013 | 0.034 | 1 | 0.854 | 0.830 | 0.114–6.043 |

Dependent variable: control group=0, study group = 1. Bold values indicate statistically significant variables. PN: parenteral nutrition; CI: confidence interval. *p<0.05.

administration were found to be independent risk factors for increased development of candidemia. However, the duration of PN treatment was not found to be a prediction risk factor in our model.

Although LOS and piperacillin–tazobactam administration were found as risk factors in this study, it is known that the factors affecting the development of candidemia may differ between patient groups depending on the chosen study design. Recent guidelines recommend the use of risk prediction tools to facilitate earlier recognition and initiation of antifungal treatment[12,13]. According to the study that used "*Candida* score," surgery, multifocal colonization, PN treatment, and severe sepsis were independent predictors of proven *Candida* infection[9]. In contrast, the sensitivity of this scoring system may differ in each patient group[13]. In this study, a significant relationship was found between *Candida* score and candidemia using the Poisson regression analysis. However, the cutoff points (high risk) for the score were not found as a significant predictor. This result means that the cutoff value of the *Candida* score should be reconsidered, especially in patients receiving PN treatment.

While broad-spectrum antibiotic was presented as a risk factor in some studies[10], the others were declared that the risk factor differs between the antibiotic groups[11,14]. In a case-control study, the previous use of antibiotics (OR 2.61; p=0.03) was found as an independent risk factor for the development of candidemia[15]. However, according to a case-comparator and 10-year study, piperacillin–tazobactam was not independently associated with BSI due to non-*C. albicans* species[14]. In contrast, in this study, the administration of piperacillin–tazobactam was determined as a significant and independent risk factor for the development of candidemia due to *C. albicans*. This result stated that the increasing use of broad-spectrum antibiotics, such as piperacillin–tazobactam, is an important risk factor especially for patients susceptible to candidemia due to PN treatment. Beyond our results, the influence of the presence of vancomycin and metronidazole with the risk of candidemia was reported[11].

According to a retrospective study, candidemia was developed after an average of 17.2 (5–74) days of administration of PN treatment. A similar result was determined in this study similar to 13 (7–29) days of the PN treatment[10]. Moreover, Luzzatti et al. reported that the elderly patients who received PN treatment had a significantly higher risk of candidemia even on the seventh day of PN treatment[11]. The fact that the mean age of our patient population (63.92 years) was close to the elderly people may explain the shorter *Candida* growth time in this study.

The LOS, duration of PN treatment, and *Candida* score were higher in patients with candidemia compared with patients without candidemia. Similar to our results, higher LOS and duration of PN treatment were also reported in patients who developed candidemia[11]. In another study whose primary outcome was the relationship between time-dependent risk factors and candidemia, LOS was greater in patients with candidemia versus control patients (36 days *versus* 13 days; p<0.001)[6]. In this study, with a similar result, statistically significant differences were found between the patients with and without candidemia groups in terms of LOS (52 days *versus* 21 days; p<0.001).

According to the study by Tsai et al., thrombocytopenia was associated with survival time in non-neutropenic patients requiring PN treatment after the onset of candidemia (p=0.006)[16]. In contrast, in this study, thrombocytopenia (<150000 cells/μL) was not determined, and it was not associated with the development of candidemia (p=0.62).

Particularly, since this is a retrospective study, it harbors some conspicuous limitations. Limited study period, small sample size, and lack of duration of antibiotic administration to evaluate the effect on the development of candidemia were the other limitations of this study.

## CONCLUSIONS

This is the first study showing that piperacillin–tazobactam, a broad-spectrum antibiotic, is a significant and independent risk factor for candidemia in non-neutropenic patients receiving PN treatment. There are some well-known risk factors including the longer hospitalization and duration of PN treatment and higher *Candida* risk score; piperacillin–tazobactam treatment should also be considered as an important risk factor. Our data suggest that randomized controlled studies should be performed to evaluate the effectiveness of candidemia prevention by restricting the use of broad-spectrum antibiotics, such as piperacillin–tazobactam, and by managing PN treatment consistent with the current guidelines.

## AUTHORS' CONTRIBUTIONS

**NY:** Conceptualization, Data curation, Methodology, Writing – original draft, Writing – review & editing. **NS:** Data curation, Investigation, Writing – original draft, Writing – review & editing. **OOK:** Data curation, Writing – original draft, Writing – review & editing. **BKC:** Investigation, Writing – original draft, Writing – review & editing. **KD:** Supervision, Visualization, Writing – review & editing. MG: Visualization, Writing – review & editing.

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# Evaluation of the factors predicting the need for intensive care of patients with COVID-19 aged above 65 years: data from an emergency department in Turkey

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## SUMMARY

**OBJECTIVE:** Individuals aged ≥65 years are more susceptible to COVID-19 disease and admission to intensive care is most notable. The scoring systems (national early warning score, quick sequential organ failure assessment, shock index) are recommended for rapid assessment of patients in emergency room conditions. The goal of our study is to evaluate scoring systems in conjunction with predictive factors of need for admission to intensive care of patients ≥65 years old with a diagnosis of COVID-19 who applied to the emergency room.

**METHODS:** Patients were divided into two groups according to evolution in the emergency room, being those who needed or not intensive care. National Early Warning Score, quick sequential organ failure assessment, shock index scores and serum biochemistry, blood count and blood gas values were evaluated from hospital information management system records.

**RESULTS:** Of the patients included in the study, 80.8% were admitted to the ward and 14.5% to the unit of intensive care. Lymphocyte count, base deficit and bicarbonate levels were lower, and the levels of C-reactive protein, lactate, D-dimer, urea and lactate dehydrogenase were higher in patients who needed intensive care. Quick sequential organ failure assessment and shock index were considered significant in the group admitted to the intensive care unit.

**CONCLUSIONS:** We recommend that quick sequential organ failure assessment and shock index be used quickly, practically and easily in predicting the need for intensive care unit in patients aged ≥65 years in emergency department diagnosed with COVID-19.

KEYWORDS: Age factors. Emergency medical services. Intensive care units.

## INTRODUCTION

Coronavirus-19 (COVID-19) disease is an infectious disease caused by severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2)[1,2]. Studies show that individuals aged ≥65 years are more susceptible to COVID-19 disease and have higher rates of hospital, intensive care, intubation, postintubation complications, and death[3-6]. Regarding the comorbid diseases, weak immune system also plays a role in increasing the sensitivity[7,8]. COVID-19, which develops with atypical symptoms, progresses to multiorgan failure within this age group[9]. It is noteworthy that admissions to the intensive care unit (ICU) are often the patients aged above 65 years[10]. A number of scoring systems have been developed to rapidly evaluate the patients with COVID-19 admitted to the emergency department. It has

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been stated that the National Early Warning Score (NEWS) in patients who are admitted to emergency department can reveal accurate results in both mortality within the hospital and hospitalization of patients from the emergency department to the ICU[11]. Quick Sequential Organ Failure Assessment (q-SOFA) is recommended as it provides a rapid prognosis to emergency department and critical care doctors and helps predict mortality[12]. The shock index (SI) can be used to predict mortality and the need for intensive care[13]. This study aims to determine the factors that predict the hospitalization of patients who aged above 65 years and diagnosed with COVID-19 in the emergency department and to evaluate those factors using the abovementioned scoring and indices.

# METHODS

## Study design

The study was planned retrospectively and was started after the approval of the ethics committee (dated: February 5, 2021, No. 2021-01). The patients with a diagnosis of COVID-19 who were hospitalized to the emergency department of the university hospital between June 2020 and February 2021 were included in this study. Demographic findings, vital signs, serum biochemistry, hemogram, and blood gas values of the patients were analyzed. The NEWS, q-SOFA score, and SI were used to assess the severity of patient with COVID-19. The patients were divided into two groups, namely, patients who need intensive care and those who do not, according to the outcome of the emergency department.

## Patients

The patients with positive polymerase chain reaction, aged above 65 years, and diagnosed with COVID-19 were included in this study. The patients aged above 65 years, trauma patients, the patients who were not diagnosed with COVID-19, and whose information could not be obtained from the system were excluded from the study.

## Laboratory analysis

Serum biochemistry analyses were performed using colorimetric method in the 501 module of the Roche Cobas 6000 device, the hemogram analyses with the electrical impedance method in the Beckman Coulter DXH 800 device, and the blood gas values were examined using ISE (ion-selective electrode) potentiometric method in the radiometer ABL 800 device.

## Statistical analysis

Mann–Whitney U test was used for numerical variables and chi-square test for comparison of categorical variables. Logistic regression analysis was applied to predict ICU admission. A base model was created using the data with statistical significance in the multivariable analysis. The DeLong test was used for a pairwise comparison of the area under the curves[14]. SPSS version 26.0 was used for statistical analysis.

# RESULTS

A total of 400 patients aged above 65 years and diagnosed with COVID-19 were included in the study. The mean age was 73 (interquartile range [IQR]: 68.0–80.75), and 229 (57.3%) patients were males. Demographic, laboratory parameters, and other characteristics of the patients are indicated in Table 1.

Comparing the groups with and without ICU hospitalization in terms of gender and the emergency department outcome, the NEWS was not significantly different between the two groups (p=0.630), while q-SOFA and SI were found to be significantly higher in the ICU group than the non-ICU groups (p<0.001), as given in Table 2.

The relationship between risk factors and mortality in patients with COVID-19 is given in Table 3.

# DISCUSSION

In a study by Lee et al.[15] in patients aged above 65 years with a diagnosis of COVID-19, the median age was 72 and the majority were females[15]. Jin et al.[16] and Jansen et al.[17] showed that elderly males are more affected by COVID-19 infection than females. Elderly male gender is more prone to COVID-19 disease[18]. The mean age (73 years) and the predominance of male gender in our study correlate with the literature. The most common comorbid diseases in the elderly are hypertension (HT), cardiovascular diseases, diabetes mellitus (DM), chronic obstructive pulmonary disease, and hyperlipidemia[19]. In our study, HT and DM were found to be underlying comorbid diseases. Most common drugs related to these diseases used by the patients are antiplatelet and angiotensin-converting enzyme (ACE) inhibitors. HT is the most common comorbid disease diagnosed among the elderly patients. It was found that the use of drugs affecting the renin–angiotensin–aldosterone (RAS) system, due to the ability of COVID-19 disease to enter the host cell by binding to ACE-2, increases the sensitivity to COVID-19 and causes viral replication[20,21]. In our study, the hospitalization rate of the patients aged above 65 years and diagnosed with COVID-19 was high. This finding is in line with many other studies in the literature[3-6]. Only 3.5% of the patients were discharged from the emergency department. It was found that the more severe the COVID-19, the higher is the mortality and the need for intensive care in the elderly patients[4]. In China and Italy, most mortality cases are with the patients aged above

**Table 1.** Demographic parameters for study population.

| | All patients (n=400) |
|---|---|
| Age | 73.0 (68.0–80.75) |
| Sex/male, n (%) | 229 (57.3) |
| Vital signs at triage | |
| Heart rate (beat/min) | 87.0 (77.0–99.0) |
| Respiratory rate | 22.0 (18.0–24.0) |
| SBP (mm Hg) | 130.5 (115.0–147.0) |
| DBP (mm Hg) | 78.0 (68.0–98.0) |
| $SO_2$ (%) | 95.0 (90.0–98.0) |
| Chronic diseases, n (%) | |
| HT | 258 (64.5) |
| DM | 115 (28.8) |
| COPD | 73(18.3) |
| CAD | 78 (19.5) |
| Stroke | 36 (9.0) |
| Cancer | 28 (7.0) |
| CHF | 33 (8.3) |
| CRD | 20 (5.0) |
| Drug use, n (%) | |
| Antiplatelet | 102 (25.5) |
| ACE inhibitors | 92 (23.0) |
| Oral anticoagulants | 81 (20.3) |
| Laboratory parameters | |
| Lymphocyte | 1.0 (0.6–1.6) |
| C-reactive protein | 7.68 (3.36–15.67) |
| D-Dimer | 474.5 (279.25–940.75) |
| aPTT | 29.0 (25.9–32.0) |
| INR | 1.12 (1.03–1.24) |
| ALT | 19.0 (12.0–29.97) |
| AST | 28.6 (19.75–42.78) |
| Urea | 49.6 (35.62–78.03) |
| Creatinine | 1.07 (0.82–1.54) |
| LDH | 326.0 (245.25–457.0) |
| Base deficit | -0.1 (-2.6, 2.3) |
| Bicarbonate | 24.2 (22.3–26.1) |
| Shock index | 0.67 (0.57–0.78) |
| NEWS≥10 (%) | 71 (17.8) |
| q-SOFA≥2 (%) | 61 (15.3) |
| Disposition, n (%) | |
| Discharge | 14 (3.5) |
| Regular ward | 323 (80.8) |
| ICU | 58 (14.5) |
| Exitus in the ED | 5 (1.25) |
| 14-Day mortality | 79 (19.8) |
| 30-Day mortality | 114 (28.5) |

SBP: systolic blood pressure; DBP: diastolic blood pressure; HT: hypertension; DM: diabetes mellitus; COPD: chronic obstructive pulmonary disease; CAD: coronary artery disease; CHF: congestive heart failure; CRD: chronic respiratory disease; ACE: angiotensin-converting enzyme; aPTT: activated partial thromboplastin time; INR: international normalized ratio; ALT: alanine transaminase; AST: aspartate aminotransferase; LDH: lactate dehydrogenase; NEWS: national early warning score; q-SOFA: quick sequential organ failure assessment; ICU: intensive care unit; ED: emergency department.

60 years. Age plays a key role in the estimation of mortality[15,22]. It is suggested that old age is a risk factor for in-hospital deaths, is more sensitive to COVID-19 disease, and can have severe courses[9]. Comparing the 14- and 30-day mortality rates, this study found that mortality increases with an increase in the length of stay in the ICU. Studies also reveal that staying in the ICU with respiratory support for a long time increases mortality[20]. Ji et al.[23] stated that the number of ICU admissions were higher in the males aged above 65 years with an underlying comorbid disease[23]. In our study, there was no difference in terms of gender and the presence and number of comorbid diseases between patients who were admitted to the ICU and those who were not.

Vital signs play a key role in the early evaluation of patients diagnosed with COVID-19 and determination of whether the patients are at risk and require ICU admission[24]. In a study conducted on 2566 patients with an average age of 63, Hao et al.[25] found that there was no difference in respiratory rate, heart rate, and mean arterial pressure between the patients hospitalized in the ICU and those who were not[25]. In our study, systolic blood pressure, low oxygen saturation, and increased heart rate were found to be significant in terms of hospitalization in the ICU. Huang et al.[10] and Wang et al.[26] reported that dyspnea symptoms were more common in the patients admitted to ICU than in nonadmitted patients; the finding that is in line with our results. Liu et al. stated that in the patients aged above 60 years with COVID-19, the lymphocyte ratio was low and the C-reactive protein (CRP) level was high. Laboratory findings of elderly patients with COVID-19 also showed lymphopenia and high levels of CRP, lactate dehydrogenase (LDH),

Table 2. Demographic findings.

| | Other, n=342 | ICU admission, n=58 | p-value |
|---|---|---|---|
| Age | 73.0 (68.0–80.0) | 75 (69.0–81) | |
| Sex: M/F, n (%) | 195 (85.2)/147 (86.0) | 34 (14.8)/24 (14.0) | 0.819 |
| Triage parameter | | | |
| SBP | 132.0 (118.0–147.25) | 116.5 (95.75–145.5) | 0.002 |
| DBP | 78.0 (69.0–87.0) | 74.0 (64.0–85.75) | 0.136 |
| Heart rate | 86.0 (77.0–98.0) | 99.5 (84.25–123.5) | <0.001 |
| Respiratory rate | 22.0 (18.0–24.0) | 22.0 (18.0–26.0) | 0.396 |
| Oxygen saturation | 95.0 (91.0–98.0) | 90.5 (81.5–97.25) | 0.004 |
| Chronic disease, n (%) | | | |
| None | 35 (77.8) | 10 (22.2) | 0.161 |
| 1 | 102 (89.5) | 12 (10.5) | |
| 2 and above | 205 (85.1) | 36 (14.9) | |
| Mortality status, n (%) | | | |
| Yes (n=127) | 87 (68.5) | 40 (31.5) | <0.001 |
| No (n=273) | 255 (93.4) | 18 (6.6) | |
| Complaints at the ED admission, n (%) | | | |
| Fever (±) | 239 (83.9)/103 (89.6) | 46 (16.1)/12 (10.4) | 0.142 |
| Cough (±) | 217 (81.3)/125 (94.0) | 50 (18.7)/8 (6) | 0.001 |
| Dyspnea (±) | 161 (95.8)/181 (78.0) | 7 (4.2)/51 (22.0) | <0.001 |
| Headache (±) | 323 (85.0)/19 (95.0) | 57 (15.0)/1 (5.0) | 0.216 |
| Fatigue (±) | 276 (83.6)/66 (94.3) | 54 (16.4)/4 (5.7) | 0.022 |
| Anosmia (±) | 331 (86.2)/11 (68.8) | 53 (13.8)/5 (31.3) | 0.052 |
| Diarrhea (±) | 325 (84.9)/17 (100) | 58 (15.1)/0 (0.0) | 0.149 |
| Joint pain (±) | 300 (84.3)/42 (95.5) | 56 (15.7)/2 (4.5) | 0.066 |
| Laboratory parameter | | | |
| Lymphocyte | 1.0 (0.7–1.6) | 0.67 (0.41–1.46) | 0.008 |
| CRP | 7.32 (3.2–14.73) | 8.69 (4.15–21.08) | 0.021 |
| Base deficit | 0.1 (-2.3, 2.43) | -1.6 (-8.95, 1.5) | 0.002 |
| Lactate | 1.6 (1.14–2.23) | 2.25 (1.47–2.97) | <0.001 |
| Bicarbonate | 24.4 (22.8–26.2) | 21.55 (16.5–25.4) | <0.001 |
| D-Dimer | 433.0 (276.0–778.0) | 982.0 (439.0–2838.0) | <0.001 |
| Urea | 48.55 (35.2–72.0) | 69.4 (42.3–117.4) | <0.001 |
| Creatinine | 1.03 (0.82–1.49) | 1.25 (0.83–1.83) | 0.098 |
| LDH | 324.5 (243.0–433.0) | 374.5 (255.0–586.0) | 0.018 |
| NEWS, n (%) | | | |
| ≤10 point | 280 (85.1) | 49 (14.9) | 0.630 |
| 11 and above | 62 (87.3) | 9 (12.7) | |
| q-SOFA, n (%) | | | |
| 0–1 point | 317 (93.5) | 22 (6.5) | <0.001 |
| 2–3 points | 25 (41.0) | 36 (59.0) | |
| Shock index | 0.65 (0.57–0.76) | 0.85 (0.69–1.14) | <0.001 |

M: male; F: female; SBP: systolic blood pressure; DBP: diastolic blood pressure; ED: emergency department; CRP: C-reactive protein; LDH: lactate dehydrogenase; NEWS: national early warning score; q-SOFA: quick sequential organ failure assessment.

**Table 3.** Association between risk factors and mortality in the patients with COVID-19 and pairwise comparisons of receiver operating characteristic curves.

| | ICU admission | | | | | | |
|---|---|---|---|---|---|---|---|
| | Univariable OR (95%CI) | p-value | Multivariable OR (95%CI) | p-value | | | |
| Age | 1.015 (0.980–1.051) | 0.405 | | | | | |
| Sex | | | | | | | |
| Female | Ref. | | | | | | |
| Male | 1.068 (0.607–1.878) | 0.820 | | | | | |
| NEWS | | | | | | | |
| 0–10 points | Ref. | | | | | | |
| ≥10 points | 0.829 (0.387–1.778) | 0.631 | | | | | |
| q-SOFA | | | | | | | |
| 0–1 point | Ref. | | | | | | |
| 2–3 points | 20.749 (10.632–40.494) | <0.001 | 19.810 (7.474–52.504) | <0.001 | | | |
| Shock index | 52.465 (14.346–191.875) | | 7.954 (1.600–39.555) | 0.011 | | | |
| Chronic disease | | | | | | | |
| None | Ref. | | | | | | |
| 1 | 0.412 (0.164–1.036 | 0.060 | | | | | |
| 2 and above | 0.615 (0.280–1.350) | 0.225 | | | | | |
| Complaints at the ED admission | | | | | | | |
| Cough | 0.278 (0.128–0.605) | 0.001 | | | | | |
| Dyspnea | 6.481 (2.860–14.686) | <0.001 | 6.420 (2.044–20.164) | 0.001 | | | |
| Fatigue | 0.310 (0.108–0.886) | 0.029 | | | | | |
| Laboratory parameter | | | | | | | |
| Lymphocyte | 1.000 (0.970–1.030) | 0.995 | | | | | |
| CRP | 1.038 (1.010–1.068) | 0.008 | | | | | |
| Base deficit | 0.896 (0.853–0.942) | <0.001 | | | | | |
| Lactate | 1.269 (1.101–1.462) | 0.001 | | | | | |
| Bicarbonate | 0.856 (0.806–0.910) | <0.001 | | | | | |
| D-Dimer | 1.000 (1.000–1.000) | 0.025 | | | | | |
| Urea | 1.012 (1.007–1.017) | <0.001 | 1.010 (1.002–1.018) | 0.012 | | | |
| LDH | 1.002 (1.000–1.003) | 0.009 | | | | | |
| Prognostic model | Area under the ROC curve (95%CI) | Pairwise analysis | | | | | |
| | | DBA | SE | 95%CI | | Z-statistic | p-value |
| | | | | Lower | Upper | | |
| Base model=Dyspnea+SI+Urea | 0.825 (0.760–0.883) | 0.058 | 0.236 | 0.022 | 0.093 | 3.180 | 0.001 |
| Base model+q-SOFA | 0.883 (0.830–0.936) | | | | | | |

ICU: intensive care unit; OR: odds ratio; CI: confidence interval; NEWS: national early warning score; q-SOFA: quick sequential organ failure assessment; ED: emergency department; CRP: C-reactive protein; LDH: lactate dehydrogenase; ROC: receiver operating characteristic; SE: standard error.

and D-dimer[4,27,28]. In our study, unlike lymphopenia, base deficit and bicarbonate were found to be low. CRP, D-dimer, urea, and LDH levels were high, whereas lactate levels were high. These results were statistically significant, which are in line with the findings of the studies in the literature.

In the patients aged above 65 years with a diagnosis of COVID-19, the NEWS gave the most accurate score for both ICU admission and mortality estimation[29]. In our study, the NEWS was not significant for both groups. Due to the additional diseases of patients aged above 65 years and the reflection of these diseases on vital signs, it has been observed that it is as high in patients who are followed up in the service without the need for intensive care as in the patients in the ICU. In a retrospective study with patients with COVID-19, q-SOFA is recommended for the prediction of respiratory failure and mortality[30]. Doğanay et al.[13] stated that the SI is a useful parameter for mortality prevention, early intervention, and hospitalization of elderly patients and patients with COVID-19 with low oxygen saturation[13]. In our study, the use of q-SOFA in patients aged above 65 years with SI was found to be more effective in the prediction of admission to the ICU.

## CONCLUSION

In age group of ³65 years, q-SOFA, SI, dyspnea, and urea elevation are effective in predicting the need for intensive care.

## AUTHORS' CONTRIBUTIONS

**CA:** Conceptualization, Data curation, Investigation, Methodology, Writing – original draft, Writing – review & editing. **MD:** Conceptualization, Formal analysis, Methodology, Writing – original draft, Writing – review & editing. **OB:** Investigation, Writing – original draft, Writing – review & editing. **GA:** Conceptualization, Investigation, Writing – original draft, Writing – review & editing. **OA:** Writing – review & editing.

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# Phobia of COVID-19 on people who aged 18 and older

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## SUMMARY

**OBJECTIVES:** Our aim was to evaluate the participants with the COVID-19 scale in order to see the effects of the COVID-19 pandemic on people, which has affected the whole world along with our country, to be able to take the necessary precautions for the current pandemic and similar pandemics and to minimize the negative aspects globally.

**METHODS:** A total of 1010 people who aged 18 and older (between the ages of 18–76) were included in the research. Besides from the personal information of people who aged 18 and older such as a city of residence, age, gender, profession, education, the number of people who live in the same house, their chronic illnesses, marital status, and the existence of a child, a 20-question phobia of COVID-19 scale was carried out. The results were 95% reliable, and their significance was evaluated to be on $p<0.05$ level.

**RESULT:** The COVID-19 Phobia Scale point for women was 54.97±14.44 while it was 51.28±14.06 for men, and between the two groups, there is a high level of significant difference ($p<0.05$) statistically, COVID-19 Phobia Scale point of people who have chronic illnesses is 56.51±15.84, meanwhile, the point of people who have no chronic illnesses was found to be 52.96±13.99, and it was detected that this difference was statistically significant ($p<0.05$).

**CONCLUSION:** Besides the COVID-19 pandemic affecting the whole society, we see that the women population and people who have chronic illnesses are going through much more fear and anxiety.

KEYWORDS: COVID-19. Pandemic. Phobia.

## INTRODUCTION

In December 2019, a pneumonia that started in Wuhan, the capital of the Hubei Province in China, which has no known factor and does not respond to the treatments, was observed. After studies were carried out, it has been understood that the illness that is named as coronavirus disease 2019 (COVID-19) (severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)) was caused by a new coronavirus, and it was transformed into an epidemic disease and took over the whole world in a terrifying manner. It is still causing a lot of people to experience psychological, organic disruptions and death[1,2].

As per the WHO's instructions, national administrations are taking various precautions against this new epidemic that threatens the global health of people spreading from China, to protect the health of citizens and to come out of the epidemic with the least damage[3]. However, despite taking strict precautions, the affected area of the epidemic and the death cases connected to it have been monitored to be increasing continuously, which causes serious concerns on an international level[4].

This study is considered to be a source of light on the literature to see the effects of the mentioned pandemic on people in our country, to be able to take the necessary precautions

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against the current pandemic and similar pandemics, and to minimize the negative aspects globally.

### Aim

This study was conducted to determine COVID-19 (SARS-CoV-2) phobia and personal characteristics related to it on people who aged 18 and older.

### Hypotheses

a) COVID-19 phobia is high in people who aged 18 and older.
b) Factors such as a city of residence, age, gender, profession, education, the number of people who live in the same house, their chronic illnesses, marital status, the existence of a child have an effect on the COVID-19 phobia of people who aged 18 and older.

### Variables

Independent variables included city of residence, age, gender, profession, education, the number of people who live in the same house, the number of chronic illnesses they have, marital status, and the existence of a child.

Dependent variables included average point of the COVID-19 Phobia Scale (C19P-S).

## METHODS

To determine the phobia of COVID-19 on people who aged 18 and older, a descriptive study was planned; after getting written permissions from writers of scales, an approval was taken from the Health Ministry and ethics committee; and after that in July 2020, the patient information were collected. The research was carried out online and 1010 people (between the ages of 18–76) who aged 18 and older were included in it. No exclusion criteria were determined.

Participants who accepted to join the research voluntarily were informed about their rights and the research properly and their "informed consent" was obtained prior to the research. All rights of the participants were respected and attention was paid to the principles of voluntarism and confidentiality.

### Data collection tools

- **Personal information form:** It is a form consisting of clauses about the city of residence, age, gender, profession, education, the number of people who live in the same house, their chronic illnesses, marital status, and the existence of a child of a person who aged 18 and older.
- **COVID-19 phobia scale:** The sample of Arpaci et al.'s research consists of 1250 people who aged 18 and older. The C19P-S is a 5-point Likert-type self-evaluation scale which was developed to evaluate the phobia that could develop against coronavirus. Scale items are evaluated between 1 "I certainly do not agree" and 5 "I certainly agree." Items 1, 5, 9, 13, 17 and 20 evaluate the Psychological subdimension; 2, 6, 10, 14, and 18 evaluate the Somatic subdimension; 3, 7, 11, 15, and 19 evaluate the Social subdimension; and 4, 8, 12, and 16 evaluate the Economic subdimension. Subdimension scores are obtained by the total score of the answers given to the items in that subdimension, meanwhile the total C19P-S score is obtained by the sum of the subdimension scores and ranges from 20–100 points. The height of the scores indicates the height in the subdimensions and general corona phobia[5].

While the data obtained in the research are evaluated, IBM SPSS statistics 22.0 program was used for statistical analyses. While evaluating the study data, besides descriptive statistical methods (i.e., mean, standard deviation, and frequency), the Student's *t* test was used for comparing normally distributed data, and the Mann–Whitney U test was used for comparing non-normally distributed data. To evaluate the correlation between data, Pearson's correlation analysis was used for normally distributed data and Spearman's correlation analysis was used for data that did not show normal distribution. The results were 95% reliable, and its significance was evaluated to be on a $p<0.05$ level.

## RESULTS

A total of 1010 people, 640 females (63.4%) and 370 males (36.6%), were included in the study. The total average age of the participants was 36.54 years and 37.95±11.12 years for men and 35.73±9.16 years for women, and there is a statistically significant difference between women and men in terms of age ($p<0.05$).

The C19P-S score was 54.97±14.44 in females and 51.28±14.06 in males, and there was a statistically high significant difference between the two groups ($p<0.05$) (Table 1).

While the C19P-S score of the participants with chronic disease (n=185) was 56.51±15.84, it was 52.96±13.99 for those without the chronic disease (n=825). There was a statistically significant difference between the two groups ($p<0.05$) (Table 1).

The married participants (n=672) had a C19P-S score of 54.28±14.50 while the unmarried (n=338) C19P-S score was 52.29±14.14, and there was a statistically significant difference between the two groups ($p<0.05$) (Table 1).

Table 1. Relationship of COVID-19 phobia scale score between groups.

| | COVID-19 phobia scale score | p-value |
|---|---|---|
| Women (n=640) | 54.97±14.44 | 0.001 |
| Men (n=370) | 51.28±14.06 | |
| People with chronic diseases (n=185) | 56.51±15.84 | 0.002 |
| People with no chronic diseases (n=825) | 52.96±13.99 | |
| Married (n=672) | 54.28±14.50 | 0.003 |
| Unmarried (n=338) | 52.29±14.14 | |
| People with a child (n=620) | 54.27±14.73 | 0.061 |
| People with no child (n=390) | 52.52±13.9 | |
| 40 and older (n=375) | 54.07±13.94 | 0.164 |
| Under 40 years old (n=635) | 53.34±14.68 | |

While the C19P-S score of the participants (n=620) with children was 54.27±14.73, it was 52.52±13.9 for those without children (n=390). There was no statistically significant difference between the two groups (p>0.05) (Table 1).

Taking the age of 40 as the limit, the C19P-S score of the participants aged 40 years and above was 54.07±13.94 (n=375), while the C19P-S score under 40 years old was 53.34±14.68 (n=635) and the difference between the two groups was not statistically significant (p>0.05) (Table 1).

When we look at the correlation between age and C19P-S score, no significant correlation was found (r=0.015; p>0.05). There was no significant correlation between the number of people living with them at home and the C19P-S score (r=0.025; p>0.05).

Among the 20 questions asked in the scale, the answer to the question "I am extremely afraid of the possibility of someone in my family getting coronavirus" was the highest with 3.71, while the answer to the question "fear of getting coronavirus worries me a lot" was the second with a score of 3.44, and the answer to the question "uncertainties about coronavirus worries me seriously" was the third with a score of 3.36 (Table 2).

## DISCUSSION

In the performed literature review, no studies were found on COVID-19 phobia except a few studies carried out with different scales. In addition, there are various studies on COVID-19-induced anxiety, sleep disorders, and depression. In the study, it was determined that the C19P-S evaluation results were statistically significantly higher for women compared to men. It is also seen in the study of Xi Liu et al.[1] (which examined the psychological situation and behavioral changes in the society of COVID-19 in China where the epidemic emerged) that women are more affected. Again, Özdin et al. found in their study investigating the rates of depression and anxiety in Turkish society during COVID-19 that women were at higher risk and female gender was an independent risk factor in this respect[6]. Again, in the study conducted by Qui J et al. in China, where the psychological situation in COVID-19 was evaluated with a large scale of participants, it was found that women were much more vulnerable to stress and were more likely to develop post-traumatic stress disorder[7]. As it can be understood from this and similar studies, it is seen that women experience stress, phobia, and anxiety more intensely and are more sensitive than men in the face of traumatic events due to them being more emotional.

In the study, the C19P-S scores of the participants with various chronic diseases were found to be significantly higher than the participants without a chronic disease. In a study that evaluates stress, anxiety, and depression conducted by Ozamiz et al. in Spain where the epidemic was severe, it was observed that people with chronic diseases had higher levels of stress, anxiety, and depression compared to participants who did not report such diseases[8]. For sure, this is an expected situation. Since the beginning of the epidemic, because both WHO and other health authorities have stated that the COVID-19 will be more severe and mortal in individuals with chronic diseases, it is understood that individuals with chronic diseases experience more fear, anxiety, and depression because they see themselves in a more risky group.

Again, in the study, it was determined that the C19P-S scores of the married participants were statistically significant compared to the unmarried participants; although the C19P-S scores of the participants with children were higher than those without children, this difference was not found significant. Again, no significant difference was found between those living alone at home and those living with more than one person in terms of scores on the C19P-S. At the same time, no significant correlation was found in the correlation assessment between the number of people the participant lives with at home and the score of the C19P-S. In the COVID-19-based panic and generalized anxiety assessment made by Islam et al. on Bangladesh society, the level of panic and anxiety was found to be significantly higher in those who were married and those living in a large family at a shared home[9]. In our study, this is similar to the higher C19P-S scores in married patients, and this may be due to an additional fear in the participant's family; however, out of 20 questions in C19P-S, "I am extremely afraid of

Table 2. COVID-19 phobia scale.

| Subdimensions | Questions | Scores |
|---|---|---|
| Psychological Subdimension | The fear of catching the coronavirus worries me a lot. | 3.44 |
| | I'm extremely afraid of the possibility of someone from my family catching the coronavirus. | 3.71 |
| | News of deaths caused by coronavirus worries me extremely. | 3.21 |
| | The uncertainties about the coronavirus worry me seriously. | 3.36 |
| | The speed of coronavirus spreading makes me panic extremely. | 3.11 |
| | I Argue (or want to) furiously with those around me because of the insensitive behavior of people toward the coronavirus. | 3.12 |
| Somatic Subdimension | I have stomach cramps because of coronavirus. | 2.01 |
| | I have chest pain because of coronavirus. | 2 |
| | My hands and feet tremble because of coronavirus. | 1.91 |
| | I experience sleeping problems because of the fear of coronavirus. | 1.91 |
| | Coronavirus makes me so nervous that I can't even do the things I normally do. | 2.24 |
| Social Subdimension | When I see coughing people, the doubt of coronavirus makes me worry extremely. | 3.14 |
| | With the doubt of coronavirus, I run away from people who sneeze. | 3.08 |
| | I'm aware that I spend too much time to clean my hands because of coronavirus. | 2.81 |
| | My social relationships are coming to a full stop for fear of getting coronavirus. | 2.94 |
| | I can't stop myself from the fear of catching coronavirus from others. | 2.81 |
| Economical Subdimension | I'm afraid of food resources running out due to coronavirus. | 2.46 |
| | I'm worried about cleaning items running out due to coronavirus. | 2.20 |
| | I stock up food in fear of coronavirus. | 1.97 |
| | After coronavirus, I'm not at ease if I don't check the supplies in the house. | 2.29 |

the possibility of a family member getting the coronavirus." getting the highest score may explain this situation. As for the participants with children, even though their C19P-S scores were higher than those without children, it was still not significant. This suggests that this may be due to the health authorities' statement that the risk in children has been low since the beginning of the epidemic or that it may be due to the nature of the participation group we studied. In the study, it was found that only 42 of the participants were living alone, while 968 people were living with more than one person. For this reason, it is thought that it would not be correct to make a clear evaluation due to a large number of differences between groups.

In the study, when we divided the patients into groups as younger than 40 years old and above, no significant difference was found between the groups in terms of C19P-S score, and when we look at the correlation between age and C19P-S score, no significant correlation was found. In terms of the evaluation of generalized anxiety, depression, and sleep disorders related to COVID-19, which was conducted by Huang et al. in China, young individuals showed a significantly higher prevalence of generalized anxiety disorder and depressive symptoms compared to the elderly[10]. In contrast, the study by Yanet Cortes-Alvarez et al. on Mexicans determined that older participants showed more stress than younger people and experienced more psychological distress due to the COVID-19 outbreak[11]. In our country, the government is taking special isolation measures for the elderly population since the beginning of the epidemic may have caused the elderly population to feel more secure, resulting in no significant difference in age in terms of C19P-S score. However, in our study, the ages of the patients were between 18–76, the average age was 36–54, and the number of elderly patients was relatively small, so we believe that it is not very right to make a crystal clear evaluation.

In this study, the answer "fear of catching coronavirus worries me extremely" given to C19P-S questions ranks second with 3.44 points, and with a point of 3.36, the answer "The uncertainties about the coronavirus worry me seriously." ranks third. We think that with the decrease in the uncertainties in the course of the coronavirus over time, the participants may experience less fear.

The limitations of this study can be stated as the fact that it consists of relatively young patients and that the participation is from regions with more urban settlements.

For this reason, we think that besides the measures to be taken for the whole society, certain measures should be taken to reduce the fear and anxiety in these potentially risky groups.

## CONCLUSIONS

We see that in addition to the COVID-19 pandemic affecting the whole society, especially the female population and people with chronic diseases experience much more fear and anxiety.

## AUTHORS' CONTRIBUTIONS

**MZ:** Conceptualization, Writing – original draft. **MK:** Conceptualization, Data curation, Formal analysis, Writing – review & editing. **NK:** Writing – original draft.

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# Outcomes associated with Hydroxychloroquine and Ivermectin in hospitalized patients with COVID-19: a single-center experience

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## SUMMARY

**OBJECTIVE:** Hydroxychloroquine and Ivermectin are advocated as potential treatments for coronavirus disease 2019 (COVID-19) despite the lack of supportive clinical evidence. In this study, outcomes associated with Hydroxychloroquine and/or Ivermectin were determined in a series of patients with confirmed COVID-19 from a single institution in Brazil.

**METHODS:** Consecutive patients admitted between March and July 2020 were retrospectively analyzed and divided into four treatment categories: no treatment (Group 0), Ivermectin only (Group I), Hydroxychloroquine only (Group II), and Hydroxychloroquine and Ivermectin (Group III). Intensive care unit admission, mechanical ventilation, and death were compared between the Groups.

**RESULTS:** A total of 230 patients were included, with the following treatment distribution: 35.2% (0), 9.1% (I), 48.3% (II), and 7.4% (III). Groups I, II, and III had the higher rates of Intensive care unit admission, mechanical ventilation, or death (0: 23.5% *versus* I: 38.1% *versus* II: 37.8% *versus* III: 70.6%, p=0.002), and the greatest mortality was found in Group III (0 *versus* III: 13.6% *versus* 35.3%, p=0.03). In the multivariate analysis, Hydroxychloroquine remained significantly associated with death (OR 3.3, 95%CI 1.1–9.6, p=0.03).

**CONCLUSION:** In a series of consecutive hospitalized patients with COVID-19, Ivermectin was not associated with improved outcomes and Hydroxychloroquine may have resulted in a harmful effect.

KEYWORDS: Coronavirus. Hydroxychloroquine. Ivermectin. Prognosis.

## INTRODUCTION

The pandemic associated with the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) continues to impose an unprecedented burden on several governments, health systems, and scientific communities around the world. As of June 22, 2021, over 179.6 million global cases of coronavirus disease-2019 (COVID-19) had been reported with more than 3 million deaths, according to the World Health Organization (WHO)[1]. As the number continues to increase, the search for effective evidence-based interventions in both prevention and treatment also continues. Social distancing, personal protective equipment, and adequate hand sanitation are still the main preventive measures, whereas appropriate respiratory support, selective steroid therapy, and management of secondary complications remain the cornerstones of treatment[2,3].

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Several medications have emerged as potential treatments for COVID-19, though there is limited supportive evidence for safety and efficacy. Most interventions have been based on nonclinical studies, observational data, and personal opinions of health care professionals[4,5]. The antimalarial hydroxychloroquine (HCQ) and antiparasitic ivermectin (IVM) are still advocated by many physicians and government officials as effective options in this context, particularly when initiated during the earlier days of symptoms[6]. The accumulated experience with these medications in other clinical scenarios has created a perception of similar safety profiles when prescribed for COVID-19. Although recent randomized trials have demonstrated the lack of benefit associated with HCQ and IVM, they remain frequently utilized in many areas where COVID-19 cases are still uncontrolled, such as Brazil and India[1,7,8]. In this study, clinical outcomes associated with HCQ and/or IVM were evaluated in a series of hospitalized patients with COVID-19 from a single institution in Brazil.

## METHODS

Consecutive patients with COVID-19 admitted between March 12 and July 8, 2020, were retrospectively analyzed using medical chart review. Only those with a positive polymerase chain reaction result for SARS-CoV-2 were included. Clinical and laboratory information were collected by four trained physicians utilizing a prespecified form with detailed instructions. The accuracy of data extraction was confirmed by a fifth physician by random evaluation of completed forms from all reviewers.

Patients were divided into four Groups based on the prescription of HCQ and IVM before or after hospitalization: no treatment (Group 0), IVM only (Group I), HCQ only (Group II), and HCQ and IVM (Group III). Treatment begins with the administration of at least one dose of IVM or HCQ after the beginning of COVID-19 symptoms. Baseline clinical and laboratory characteristics, in addition to in-hospital outcomes, were compared between the Groups. Adverse outcomes included intensive care unit (ICU) admission rate, mechanical ventilation (MV) requirement, and death.

For statistical analysis, Stata® version 11.0 software was used. Categorical variables were analyzed with $\chi^2$ and Fisher's exact tests. All continuous variables were non-normally distributed using Shapiro–Wilk test and were expressed as median and interquartile range (IQR) (25th–75th percentile). The collected data were evaluated by the Wilcoxon–Mann–Whitney and Kruskal–Wallis tests. To evaluate association between continuous variables and mortality, the graphical models were represented by predicted probability plots. Significant variables in the univariate analysis were included in a multivariate logistic regression model to determine independent predictors of death. A $p<0.05$ was considered significant. The study conforms to the guidelines of the Declaration of Helsinki and was approved by the hospital's ethics committee.

## RESULTS

During the study period, 230 patients with confirmed COVID-19 who were admitted at our institution were included in the analysis. Patients were predominantly males with a median age of 68 years (IQR 54–82) and an elevated prevalence of hypertension and diabetes, despite a low frequency of underlying pulmonary disease and current or previous tobacco use. The median symptom duration before admission was 6 days (IQR 3–9), and overall oxygen saturation at room temperature was 93% (IQR 91–96). Complete clinical and laboratory baseline characteristics are listed in Table 1.

Table 1. Baseline characteristics according to treatment Group.

| Baseline characteristics | Total, n=230 | IVM and/or HCQ during hospitalization | | | | p-value[a] |
|---|---|---|---|---|---|---|
| | | No (0), n=81 | IVM only (I), n=21 | HCQ only (II), n=111 | IVM+HCQ (III), n=17 | |
| Age, median (IQR), years | 68 (54–82) | 77 (61–87) | 68 (56–79) | 63 (50–74)[c] | 76 (62–83) | 0.003 |
| Male, n (%) | 132 (57.4) | 40 (49.4) | 13 (61.9) | 69 (62.2) | 10 (58.8) | 0.34 |
| BMI, median (IQR), kg/m$^2$ | 26.4 (23.9–30.1) | 25 (22.9–28.2) | 26.9 (24.8–32.0)[c] | 28 (24.9–30.3)[c] | 28.6 (23.8–30.9) | 0.008 |
| Medical history, n (%) | | | | | | |
| Hypertension | 119 (51.7) | 45 (55.6) | 12 (57.1) | 54 (48.7) | 8 (47.1) | 0.73 |
| Diabetes | 67 (29.1) | 29 (35.8) | 5 (23.8) | 29 (26.1) | 4 (23.5) | 0.43 |
| CVD | 34 (14.8) | 16 (19.8) | 3 (14.3) | 14 (12.6) | 1 (5.9) | 0.38 |

Continue...

Table 1. Continuation.

| Baseline characteristics | Total, n=230 | IVM and/or HCQ during hospitalization | | | | p-value[a] |
|---|---|---|---|---|---|---|
| | | No (0), n=81 | IVM only (I), n=21 | HCQ only (II), n=111 | IVM+HCQ (III), n=17 | |
| Heart failure or LVD | 5 (2.17) | 2 (2.5) | 0 | 3 (2.7) | 0 | 0.79 |
| Asthma or COPD | 25 (10.9) | 11 (13.6) | 2 (9.5) | 9 (8.1) | 3 (17.7) | 0.51 |
| Cancer | 31 (13.5) | 15 (18.5) | 4 (19.1) | 11 (9.9) | 1 (5.9) | 0.22 |
| Current or prior tobacco use | 24 (10.4) | 10 (12.4) | 4 (19.1) | 7 (6.3) | 3 (17.7) | 0.18 |
| Symptom duration before admission, median (IQR), days | 6 (3–9) | 7 (3–9) | 7 (5–10) | 6 (3–8) | 6 (5–10) | 0.21 |
| Oxygen saturation on ambient air, median (IQR), % | 93 (91–96) | 94 (91–96) | 94 (91–96) | 93 (90–95) | 92 (90–95) | 0.50 |
| Systolic BP, median (IQR), mm Hg | 130 (119–145) | 130 (119–146) | 128 (115–140) | 132 (120–145) | 120 (119–130) | 0.29 |
| Blood test, median (IQR) | | | | | | |
| Leukocyte count, cells/mm$^3$ | 5905 (4590–8290) | 6140 (4590–10110) | 5850 (4850–8350) | 5800 (4360–7860) | 6320 (5140–8050) | 0.75 |
| Lymphocyte count, cells/mm$^3$ | 928 (669–1250) | 920 (645–1282) | 708 (541–1028) | 958 (680–1250) | 928 (741–1115) | 0.54 |
| Hemoglobin, mg/dL | 13.4 (12–14.6) | 12.9 (11.1–14.1) | 14 (11–14.6)[c] | 13.5 (12.5–14.9) | 13.2 (12.3–14.6) | 0.015 |
| Platelet count, ×10$^3$/mm$^3$ | 177.5 (144–233) | 191 (148–240) | 171 (132–258) | 174 (138–219) | 175 (145–205) | 0.55 |
| C-reactive protein, mg/mL | 6.65 (3.3–14.3) | 6.1 (2.4–14) | 6.2 (4.8–13.2) | 7.7 (3.3–15) | 7 (4.7–14.3) | 0.59 |
| D-Dimer, ng/mL | 778 (457–1418) | 877 (550–1994) | 795 (486–1339) | 629 (415–1181) | 807 (505–1262) | 0.16 |
| hs-Troponin I, pg/mL | 11 (11–24.5) | 12 (11–48) | 11 (11–15.5)[c] | 11 (11–13)[c] | 11 (11–12)[c] | 0.04 |
| Creatinine, mg/dL | 0.9 (0.7–1.1) | 0.9 (0.7–1.2) | 0.9 (0.8–1.0) | 0.9 (0.7–1.1) | 1.0 (0.6–1.2) | 0.99 |
| Total chest CT opacities, median (IQR), (%)[b] | 15.8 (6.7–30.4) | 14.8 (4.4–31.9) | 20.2 (5.2–41.3) | 15.9 (9.2–27.7) | 16.2 (9.0–23.5) | 0.88 |
| Previous antithrombotics, n (%) | | | | | | |
| Antiplatelets | 35 (15.2) | 17 (21) | 4 (19.1) | 11 (9.9) | 3 (17.7) | 0.14 |
| Anticoagulants | 28 (12.2) | 14 (17.3) | 2 (9.5) | 11 (9.9) | 1 (5.9) | 0.44 |
| Azithromycin, n (%) | 182 (79.1) | 48 (59.3) | 14 (66.7) | 103 (92.8)[c] | 17 (100)[c] | <0.001 |
| Hydroxychloroquine, n (%) | | | | | | |
| Total | 128 (55.7) | 0 | 0 | 111 (100) | 17 (100) | – |
| First 7 days of symptoms | 79 (34.3) | NA | NA | 68 (61.3) | 11 (64.7) | – |
| Ivermectin, n (%) | | | | | | |
| Total | 38 (16.5) | 0 | 21 (100) | 0 | 17 (100) | – |
| First 7 days of symptoms | 15 (6.5) | NA | 8 (38.1) | NA | 11 (64.7) | – |
| Symptom duration before treatment, median (IQR), days | 7 (4–9) | NA | 7 (5–10) | 6 (3–8) | 6 (5–10) | 0.21 |

[a]p<0.05 indicates statistical significance (bold values); [b]n=200; [c]Significant difference when compared to Group 0. BMI: body mass index; BP: blood pressure; COPD: chronic obstructive pulmonary disease; CT: computed tomography; CVD: cardiovascular disease; hs: high-sensitivity; HCQ: Hydroxychloroquine; IQR: interquartile range; IVM: Ivermectin; NA: not applicable.

Of the 230 patients, 21 (9.1%) received only IVM (Group I), 111 (48.2%) were treated with HCQ (Group II), and 17 (7.4%) were prescribed both drugs (Group III). The remaining 81 (35.2%) patients did not receive any of the two medications (Group 0). The median age, body mass index (BMI), and clinical variables, such as symptom duration and oxygen saturation on ambient air, were similar among all Groups. Patients in Group 0 were significantly older and with a lower BMI. On admission, those who did not receive either IVM or HCQ presented with higher levels of troponin and lower hemoglobin values. The remaining laboratory parameters and pulmonary disease burden on chest computed tomography were not significantly different. Compared to Group 0, the frequency of azithromycin use was significantly greater among patients in Groups II and III.

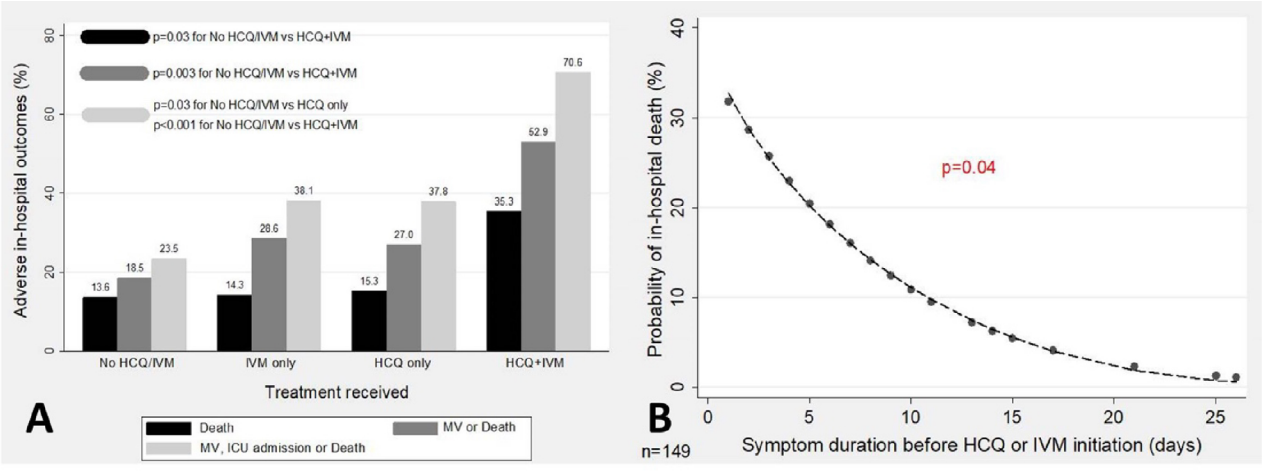
During the hospitalization period, 37 (16.1%) patients died. The median length of stay was nine days (IQR: 5–20), 72 (31.3%) patients required ICU admission, and 47 (20.4%) were treated with MV. The combined outcome of death, ICU admission, and MV was significantly higher in Groups II and III compared to Group 0. Group III also presented the greatest mortality rate (35.3%). Outcomes associated with each Group are represented in Figure 1A.

On admission, the predictors of subsequent in-hospital death in the univariate analysis included age, previous cardiovascular disease, symptom duration, oxygen saturation, systolic blood pressure, C-reactive protein, and D-dimer levels. When IVM and HCQ were added to the multivariate model, only age (OR 1.15, 95%CI 1.1–1.2, p<0.001), HCQ (OR 3.3, 95%CI 1.1–9.6, p=0.03), and C-reactive protein (OR 1.1, 95%CI 1.0–1.2, p=0.02) remained significantly associated with death. An inverse relationship was found between symptom duration before treatment initiation and death in Groups I, II, and III (Figure 1B).

## DISCUSSION

In this single–center observational study, neither IVM nor HCQ was associated with improved outcomes among hospitalized patients with COVID-19. As randomized trials continue to be published in this field, it remains important to reassess the results of practice patterns that have been adopted since the initial stages of the pandemic. Similarly to IVM and HCQ, many other interventions such as zinc, azithromycin, and favipiravir have been advocated for early treatment of the disease despite the lack of supportive clinical evidence. Recommendations are largely based on *in vitro* studies, observational data, and personal opinion, with subsequent extrapolation of theoretical clinical benefits[4,9].

Almost 18 months after the beginning of the pandemic, a variety of randomized trials have studied HCQ in different clinical scenarios and disease severities. Reis et al. randomized 685 outpatients in the first week of symptoms to either HCQ, lopinavir–ritonavir, or placebo and evaluated the effects of each intervention on admission rates or death. The study was stopped early after the first interim analysis for futility of both treatment arms. In addition, no differences were found in virological clearance rates or time to symptom




HCQ: Hydroxychloroquine; ICU: intensive care unit; IVM: Ivermectin; MV: mechanical ventilation.

Figure 1. (A) In-hospital outcomes associated with hydroxychloroquine and ivermectin use in patients with COVID-19. (B) Relationship between symptom duration before Hydroxychloroquine and/or Ivermectin prescription and subsequent probability of death.

resolution[10]. These results contradict the hypothesis that HCQ may be effective in the early stages of the infection, by limiting viral replication.

In a randomized trial among hospitalized patients with severe disease, Réa-Neto et al. reported poorer outcomes among those treated with HCQ. Similarly to our results, a high rate of azithromycin use was also seen in the intervention arm (96.2%). Patients who received HCQ were at increased risk of developing a worsening in clinical status, renal dysfunction, or requiring MV[11]. A recent meta-analysis of 28 randomized clinical trials evaluating mortality outcomes associated with HCQ has supported these results, suggesting that HCQ is associated with increased death rates in patients with COVID-19[12].

Fewer randomized trials evaluating IVM in COVID-19 have been reported, although small publications with significant methodological issues are presently available[2]. A trial published by López-Medina et al. addressed many of the previous limitations and analyzed the effect of IVM on symptom resolution among 400 outpatients with COVID-19. A 5-day course of IVM was not associated with better outcomes, although treatment was begun on the first week of symptoms[13]. As such, IVM is currently recommend by many infectious disease organizations as an option for COVID-19 only within the scope of a clinical trial[2,3].

Our study demonstrates comparable results, though outside the boundaries of a clinical trial. Despite the greater median age, patients who did not receive IVM or HCQ during hospitalization (Group 0) are much better than those in Groups I, II, and III. Notably, death, ICU admission, and MV rates were greatest among those treated with HCQ (Groups II and III), suggesting a neutral effect of isolated IVM use, but a potentially detrimental impact of HCQ, especially when combined with IVM. Even in the multivariate analysis, HCQ persisted as an independent predictor of in-hospital death. The potential adverse cardiovascular and renal effects promoted by HCQ should be appreciated and may contribute to clinical deterioration[14]. Symptom duration before treatment initiation did not improve the results and even demonstrated an inverse relationship with subsequent mortality.

This study has limitations. The retrospective and single-center nature of the results should be regarded as hypothesis generating and is not free from potential biases. The data were extracted since admissions, which occurred in the beginning of the pandemic in Brazil, when treatments of uncertain benefit were more likely to be offered to sicker patients. Nevertheless, when adjusting the analysis to other determinants of clinical severity, HCQ remained associated with death. Among those in Groups I, II, and III who were admitted to the ICU and required MV, it was not established if the medications were begun after the end points occurred. Still, median symptom duration upon admission was similar to the time of disease progression before treatment initiation, suggesting that the drugs were primarily prescribed earlier than the evaluated outcomes. Finally, the influence on outcomes of other potential interventions, such as antibiotics, tocilizumab, and corticosteroids, which may have been utilized during hospitalization, must be considered.

## CONCLUSIONS

During any pandemic, despite the eagerness to discover an effective and safe treatment in a timely manner, the standards and processes of evidence-based medicine must be followed. Several treatments for COVID-19 have been widely adopted without regarding the necessary steps of clinical research. In the current study, IVM was not associated with improved outcomes and HCQ was related to increased mortality rates. In the future, results of randomized controlled trials should be considered before the adoption of new interventions of unknown clinical benefit, especially when potential harms cannot be excluded.

## AUTHORS' CONTRIBUTIONS

**RMF:** Conceptualization, Data curation, Formal analysis, Writing – review & editing. **RWB:** Supervision, Data curation, Formal analysis. **PPNS:** Data curation, Writing – review & editing. **JMF:** Conceptualization, Writing – review & editing. **RACL:** Conceptualization, Supervision, Writing – review & editing.

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# COVID-19 in Turkish health care workers practicing chest medicine

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## SUMMARY

**OBJECTIVE:** This study aimed to evaluate the data of Turkish health care workers practicing chest medicine on their coronavirus disease 2019 (COVID-19) status and related parameters.

**METHODS:** This descriptive study included online survey data that the Turkish Thoracic Society conducted with its members in two phases starting in June and December 2020. The 33-item survey included demographic data, smoking status, the presence of any chronic diseases, occupation, working status, and non–work-related and work-related COVID-19 exposure characteristics.

**RESULTS:** Of 742 responses, 299 (40.3%) reported that they had contracted COVID-19. The second survey detected a higher frequency of health care workers who had contracted COVID-19 (12.1% *versus* 57.4%, p<0.001) than the first survey. The analysis of the association between study parameters and COVID-19 in health care workers using logistic regression revealed statistical significance with working at the onset of the outbreak (OR 3.76, 95%CI 1.09–12.98, p=0.036), not working at the time of survey (OR 5.69, 95%CI 3.35–9.67, p<0.001), COVID-19 history in colleagues (OR 2.27, 95%CI 1.51–3.41, p<0.001), any non–work-related COVID-19 exposure (OR 4.72, 95%CI 2.74–8.14, p<0.001), COVID-19 exposure at home (OR 6.52, 95%CI 3.52–12.08, p<0.001), and COVID-19 history in family members (OR 8.16, 95%CI 5.52–12.08, p<0.001) after adjusting for age and sex. The study also observed an inverse relationship between the use of aprons and goggles and COVID-19 in health care workers.

**CONCLUSION:** Occupational and nonoccupational characteristics are related to COVID-19 in health care workers practicing chest medicine. Therefore, active surveillance to detect health care workers contracting COVID-19 and to document and control occupational and nonoccupational risks should be provided.

KEYWORDS: COVID-19. Occupational health. Health personnel. Occupational medicine. Occupational diseases.

## INTRODUCTION

Coronavirus disease 2019 (COVID-19) emerged toward the end of 2019 and was declared a pandemic by the World Health Organization (WHO) on March 11, 2020, due to its rapid global spread[1]. However, the risk of infection has not been the same for all people. Indeed, workers with essential jobs, also called frontline workers, faced a higher risk than the general population during the pandemic[2]. Of those, health care workers (HCWs) have encountered occupational risks related to COVID-19[3].

In Turkey, the Minister of Health officially announced the first COVID-19 diagnosis on March 11, 2020[4]. Since then, the demand for health care services has progressively increased while the number of cases has grown[5], similar to the situation in other countries. At the initial phase of the outbreak, the Ministry of Health of Turkey defined a pandemic referral hospital as a hospital with a tertiary intensive care unit and employing specialists with at least any two specialties of internal medicine, infectious diseases, and chest medicine[4,6]. Thus, being among

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the essential members of the health care services during the outbreak in Turkey meant HCWs practicing chest medicine have faced occupational risks and contracted COVID-19 since the early days of the pandemic. The Turkish Thoracic Society (TTS), as one of the principal societies for Turkish HCWs working in chest medicine, has asked its members about their COVID-19 status and related occupational and nonoccupational characteristics via online surveys. This study aimed to evaluate COVID-19 status and related parameters of Turkish HCWs practicing chest medicine through the data collected by the TTS.

# METHODS

## Study design, study population, and data collection

This descriptive study included the data obtained by the online surveys which the TTS conducted with its members in two consecutive phases to monitor their COVID-19 status and related parameters. This study was performed in accordance with the principles of the Declaration of Helsinki. The study protocol was approved by the Duzce University Ethics Board for Noninterventional Health Research (Decision No. 2021/37).

The 33-item survey prepared by the Occupational Lung Diseases Working Group of TTS included demographic information, smoking status, the presence of any chronic diseases, occupation, current working status, working status at the onset of the outbreak, and the characteristics of work-related and non–work-related COVID-19 exposure. The characteristics of non–work-related COVID-19 exposure included the place (home or other) of exposure and if any household member had contracted COVID-19. The characteristics of work-related COVID-19 exposure included the status of work-related COVID-19 exposure according to hospital division (outpatient clinics, wards, COVID-19 triage area, intensive care unit, emergency department, and other departments), any exposure to secretions from infected patients, COVID-19 history in colleagues, and the use of personal protective equipment (PPE), namely, disposable gloves, surgical masks, respirators, facial protectors, goggles, aprons, and gowns. The participants were asked if they contracted COVID-19. The HCWs contracted COVID-19 were also questioned about the symptom status, types of symptoms, the date, and method (i.e., polymerase chain reaction [PCR], serology, and clinical and/or radiological) of COVID-19 diagnosis. In addition, the second survey asked if the respondent had participated in the initial survey.

The web links to the online surveys were sent via email by the TTS on June 1, 2020, during the first phase, and on December 8, 2020, during the second phase. The TTS members receiving the email totaled 6,103 and 6,325 in June 2020 and December 2020, respectively. The first survey remained open for 5 weeks, with four reminder emails sent weekly. The second survey remained open until the end of January 2021, and seven reminders were sent to the members. The analysis excluded duplicate records resulting from re-sent answers and second survey responses reporting prior participation in the first survey. The number of new diagnoses of COVID-19 cases per week in Turkey was derived from the WHO COVID-19 Dashboard[7].

## Statistical analysis

The descriptive statistics were presented as mean± standard deviation or median and minimum–maximum for continuous variables and as numbers and percentages for categorical variables. The chi-square test compared categorical variables. Crude and age- and sex-adjusted logistic regression analyses evaluated the relationship between parameters and COVID-19 status, and the odds ratios (ORs) with a 95% confidence interval (95% CI) values were calculated. The type I error was accepted as 0.05 for all analyses. All statistical analyses were performed using IBM SPSS for Windows version 22.0 (IBM Corp., Armonk, NY, USA).

# RESULTS

Of 868 responses, 4 duplicates and 122 responses to the second survey reporting prior participation to the first survey were excluded. In total, 742 responses (280 in the first survey and 462 in the second survey) were included, and 475 (64.0%) were females. The median age of 716 participants reported their age was 43 (min–max, 22–73). The number of participants who reported their home province was 703 (94.7%). Of those respondents, 209 (29.7%) were from Istanbul, 108 (15.4%) from Ankara, and 70 (10.0%) from Izmir. The total number of participants reporting that they had contracted COVID-19 was 299 (40.3%). Compared to the first survey, a higher frequency of HCWs contracted COVID-19 was detected in the second survey (12.1% *versus* 57.4%, p<0.001).

Figure 1 shows the weekly distribution of 278 (93.0%) HCWs who contracted COVID-19 and reported the date of diagnosis, together with the weekly number of new diagnoses of COVID-19 cases in Turkey. The highest number of diagnoses stood at 30 in the week of November 23, 2020.

In the comparison of demographic and clinical characteristics between HCWs who had contracted COVID-19 and HCWs without a history of COVID-19 (Table 1), the characteristics with significantly higher frequency in HCWs having contracted COVID-19 were having comorbid asthma,

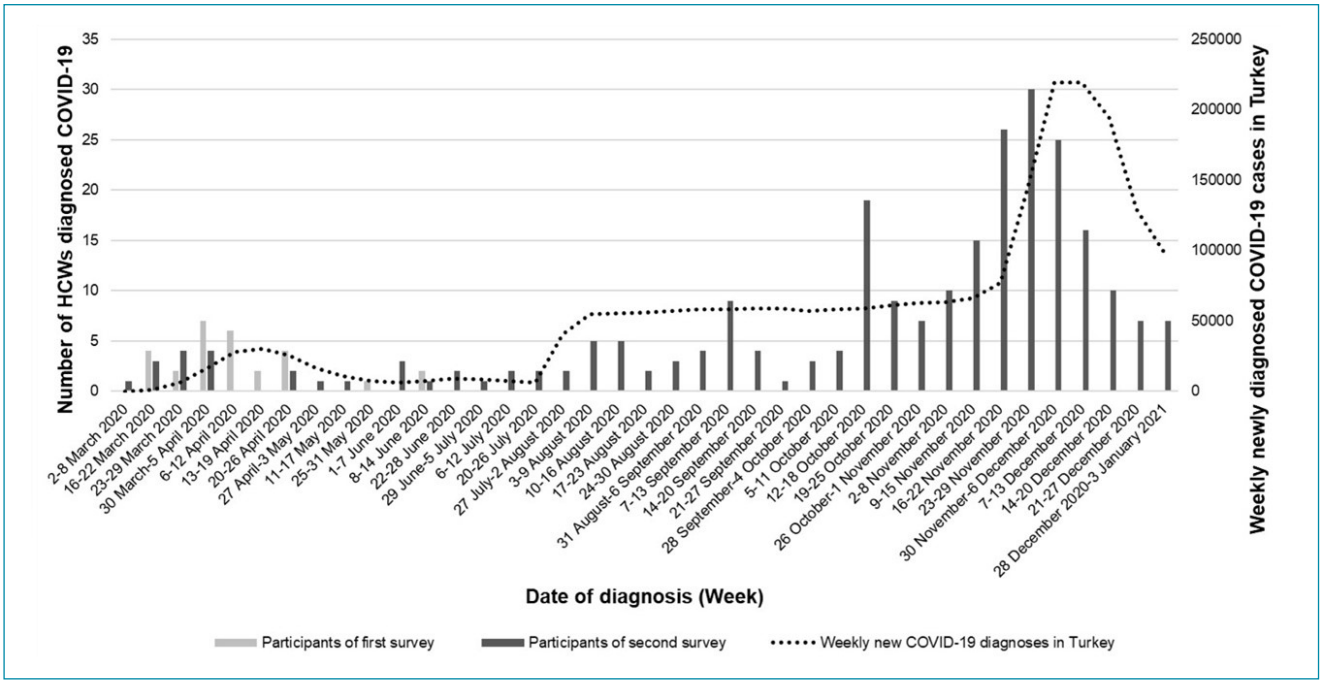


Figure 1. COVID-19: coronavirus disease 2019; HCW: healthcare worker. The distribution of HCWs with COVID-19 according to the week of diagnosis. Twenty-one participants who did not report the date of COVID-19 diagnosis were not included.

non–work-related COVID-19 exposure, COVID-19 exposure at home, COVID-19 history in household members, working at the onset of the outbreak, not working at the time of survey, and COVID-19 history in colleagues. There was also a statistically significant difference according to the smoking status. In terms of PPE, the use of aprons and goggles proved lower in HCWs with COVID-19. Similar comparisons were performed for each survey phase, and the results are presented in Table 1.

In terms of symptoms, 177 (59.2%) HCWs with COVID-19 reported that their infection was asymptomatic. The frequencies of symptoms were 70 (23.4%) for a cough, 66 (22.1%) for headache, 59 (19.7%) for loss of smell and/or taste, 47 (15.7%) for fever, 42 (14.0%) for nasal congestion and/or rhinorrhea, 40 (13.4%) for shortness of breath, 29 (9.7%) for chest pain, 21 (7.0%) for nausea/vomiting, 19 (6.4%) for diarrhea, and 16 (5.4%) for muscle or joint pain. The diagnostic method was PCR in 215 (71.9%) and positive serology in 40 (13.4%), but 44 (14.7%) were diagnosed clinically and/or radiologically.

The association between selected parameters and COVID-19 in HCWs underwent evaluation with crude and age- and sex-adjusted logistic regression analysis, and working at the onset of the outbreak (OR 3.76, 95%CI 1.09–12.98), not working at the time of survey (OR 5.69, 95%CI 3.35–9.67), COVID-19 history in colleagues (OR 2.27, 95%CI 1.51–3.41), any non–work-related COVID-19 exposure (OR 4.72, 95%CI 2.74–8.14), COVID-19 exposure at home (OR 6.52, 95%CI 3.52–12.08), and COVID-19 history in family members (OR 8.16, 95%CI 5.52–12.08) bore a significant relationship to COVID-19 in HCWs after adjusting for age and sex (Table 2). When never-smoker respondents were accepted as the reference, smoking was found to be inversely related to COVID-19 in HCWs (OR 0.38, 95%CI 0.23–0.63). The types of PPE significantly associated with lower COVID-19 infection in HCWs were wearing aprons and goggles after the adjustment.

## DISCUSSION

The number of HCWs contracting COVID-19 has increased globally during the pandemic, in line with the total number of infected people. In Figure 1, the peak in weekly diagnoses of HCWs corresponded to that of new COVID-19 diagnoses in Turkey during November and December 2020. Lan et al.[8] also found a relationship between COVID-19 infection rates in HCWs and the infection rates in their residential community. Wu et al.[9] evaluated HCW and general population infection data in Ireland and demonstrated a close relationship. The findings of this study, similar to those in the wider literature, point to the importance of community-level measures together with workplace measures to protect HCWs.

According to the findings, occupational characteristics, including working at the onset of the outbreak, not working at the time of the survey, and COVID-19 history in colleagues,

Table 1. Comparison of demographic and clinical characteristics between HCWs contracted COVID-19 and HCWs without COVID-19 history.

| Characteristics | First survey | | | Second survey | | | Total | | |
|---|---|---|---|---|---|---|---|---|---|
| | HCWs contracted COVID-19 (n=34) | HCWs without COVID-19 history (n=246) | p-value | HCWs contracted COVID-19 (n=265) | HCWs without COVID-19 history (n=197) | p-value | HCWs contracted COVID-19 (n=299) | HCWs without COVID-19 history (n=443) | p-value |
| Age, median (min–max)* | 44.5 (26–65) | 43 (23–73) | 0.534† | 42 (22–64) | 43 (23–70) | 0.253† | 43 (22–65) | 43 (23–73) | 0.367† |
| Sex, n (%) | | | 0.711‡ | | | 0.337‡ | | | 0.949‡ |
| Female | 22 (64.7) | 167 (67.9) | | 169 (63.8) | 117 (59.4) | | 191 (63.9) | 284 (64.1) | |
| Male | 12 (35.3) | 79 (32.1) | | 96 (36.2) | 80 (40.6) | | 108 (36.1) | 159 (35.9) | |
| Smoking status, n (%) | | | 0.765‡ | | | 0.009‡ | | | 0.002‡ |
| Never smoker | 24 (70.6) | 158 (64.2) | | 190 (71.7) | 128 (65.0) | | 214 (71.6) | 286 (64.6) | |
| Ex-smoker | 5 (14.7) | 43 (17.5) | | 54 (20.4) | 35 (17.8) | | 59 (19.7) | 78 (17.6) | |
| Current smoker | 5 (14.7) | 45 (18.3) | | 21 (7.9) | 34 (17.3) | | 26 (8.7) | 79 (17.8) | |
| Any comorbidity, n (%) | 11 (32.4) | 75 (30.5) | 0.825‡ | 97 (36.6) | 59 (29.9) | 0.135‡ | 108 (36.1) | 134 (30.2) | 0.094‡ |
| Comorbid hypertension, n (%) | 3 (8.8) | 29 (11.8) | 0.778§ | 43 (16.2) | 24 (12.2) | 0.222‡ | 46 (15.4) | 53 (12.0) | 0.179‡ |
| Comorbid diabetes mellitus, n (%) | 1 (2.9) | 12 (4.9) | 1.000§ | 17 (6.4) | 13 (6.6) | 0.937‡ | 18 (6.0) | 25 (5.6) | 0.829‡ |
| Comorbid coronary artery disease, n (%) | 0 | 5 (2.0) | 1.000§ | 9 (3.4) | 3 (1.5) | 0.211‡ | 9 (3.0) | 8 (1.8) | 0.282‡ |
| Comorbid hyperlipidemia, n (%) | 1 (2.9) | 6 (2.4) | 0.600§ | 12 (4.5) | 11 (5.6) | 0.606‡ | 13 (4.3) | 17 (3.8) | 0.729‡ |
| Comorbid cerebrovascular accident, n (%) | 1 (2.9) | 3 (1.2) | 0.406§ | 1 (0.4) | 3 (1.5) | 0.317§ | 2 (0.7) | 6 (1.4) | 0.485§ |
| Comorbid cancer, n (%) | 2 (5.9) | 4 (1.6) | 0.157§ | 4 (1.5) | 3 (1.5) | 1.000§ | 6 (2.0) | 7 (1.6) | 0.664‡ |
| Comorbid COPD, n (%) | 0 | 2 (0.8) | 1.000§ | 2 (0.8) | 1 (0.5) | 1.000§ | 2 (0.7) | 3 (0.7) | 1.000§ |
| Comorbid asthma, n (%) | 4 (11.8) | 20 (8.1) | 0.510§ | 33 (12.5) | 14 (7.1) | 0.060‡ | 37 (12.4) | 34 (7.7) | 0.033‡ |
| Comorbid thyroid disease, n (%) | 1 (2.9) | 8 (3.3) | 1.000§ | 12 (4.5) | 3 (1.5) | 0.071‡ | 13 (4.3) | 11 (2.5) | 0.159‡ |
| Comorbid rheumatic disease, n (%) | 1 (2.9) | 6 (2.4) | 0.600§ | 6 (2.3) | 5 (2.5) | 1.000§ | 7 (2.3) | 11 (2.5) | 0.902‡ |
| Comorbid liver disease, n (%) | 0 | 0 | – | 2 (0.8) | 2 (1.0) | 1.000§ | 2 (0.7) | 2 (0.5) | 1.000§ |
| Household size, n (%) | | | 0.550§ | | | 0.637‡ | | | 0.671‡ |
| Single | 2 (5.9) | 25 (10.2) | | 32 (12.1) | 21 (10.7) | | 34 (11.4) | 46 (10.4) | |
| Family | 32 (94.1) | 221 (89.8) | | 233 (87.9) | 176 (89.3) | | 265 (88.6) | 397 (89.6) | |
| Any household member older than 60 years of age, n (%)* | 12 (37.5) | 55 (24.9) | 0.131‡ | 48 (20.6) | 40 (22.7) | 0.604‡ | 60 (22.6) | 95 (23.9) | 0.701‡ |
| Any non–work-related COVID-19 exposure, n (%) | 4 (11.8) | 5 (2.0) | 0.003§ | 51 (19.2) | 15 (7.6) | <0.001‡ | 55 (18.4) | 20 (4.5) | <0.001‡ |
| Non–work-related COVID-19 exposure at home, n (%) | 3 (8.8) | 3 (1.2) | 0.025§ | 49 (18.5) | 11 (5.6) | <0.001‡ | 52 (17.4) | 14 (3.2) | <0.001‡ |
| Non–work-related COVID-19 exposure at other settings, n (%) | 1 (2.9) | 2 (0.8) | 0.323§ | 1 (0.4) | 4 (2.0) | 0.169§ | 2 (0.7) | 6 (1.4) | 0.485§ |
| COVID-19 history in any household member, n (%) | 9 (26.5) | 13 (5.3) | <0.001§ | 134 (50.6) | 33 (16.8) | <0.001‡ | 143 (47.8) | 46 (10.4) | <0.001‡ |
| Occupation, n (%) | | | 0.054§ | | | 0.821‡ | | | 0.279‡ |
| Physician | 34 (100.0) | 221 (89.8) | | 210 (79.5) | 155 (78.7) | | 244 (81.9) | 376 (84.9) | |
| Other | 0 | 25 (10.2) | | 54 (20.5) | 42 (21.3) | | 54 (18.1) | 67 (15.1) | |

Continue...

Table 1. Continuation.

| Characteristics | First survey | | | Second survey | | | Total | | |
|---|---|---|---|---|---|---|---|---|---|
| | HCWs contracted COVID-19 (n=34) | HCWs without COVID-19 history (n=246) | p-value | HCWs contracted COVID-19 (n=265) | HCWs without COVID-19 history (n=197) | p-value | HCWs contracted COVID-19 (n=299) | HCWs without COVID-19 history (n=443) | p-value |
| Working status at the onset of the outbreak, n (%) | | | 0.372§ | | | 0.295§ | | | 0.019‡ |
| Not working | 0 | 12 (4.9) | | 3 (1.1) | 5 (2.5) | | 3 (1.0) | 17 (3.8) | |
| Working | 34 (100.0) | 234 (95.1) | | 262 (98.9) | 192 (97.5) | | 296 (99.0) | 426 (96.2) | |
| Working status at the time of survey, n (%) | | | 0.136§ | | | <0.001‡ | | | <0.001‡ |
| Not working | 4 (11.8) | 13 (5.3) | | 60 (22.6) | 7 (3.6) | | 64 (21.4) | 20 (4.5) | |
| Working | 30 (88.2) | 233 (94.7) | | 205 (77.4) | 190 (96.4) | | 235 (78.6) | 423 (95.5) | |
| Any work-related COVID-19 exposure, n (%) | 26 (76.5) | 160 (65.0) | 0.186‡ | 151 (57.0) | 120 (60.9) | 0.396‡ | 177 (59.2) | 280 (63.2) | 0.271‡ |
| Work-related COVID-19 exposure in outpatient clinics, n (%) | 4 (11.8) | 42 (17.1) | 0.434‡ | 52 (19.6) | 52 (26.4) | 0.085‡ | 56 (18.7) | 94 (21.2) | 0.434‡ |
| Work-related COVID-19 exposure in wards, n (%) | 15 (44.1) | 78 (31.7) | 0.150‡ | 70 (26.4) | 35 (17.8) | 0.028‡ | 85 (28.4) | 113 (25.5) | 0.378‡ |
| Work-related COVID-19 exposure in COVID-19 triage area, n (%) | 1 (2.9) | 19 (7.7) | 0.485§ | 12 (4.5) | 10 (5.1) | 0.784‡ | 13 (4.3) | 29 (6.5) | 0.204‡ |
| Work-related COVID-19 exposure in intensive care unit, n (%) | 4 (11.8) | 20 (8.1) | 0.510§ | 28 (10.6) | 25 (12.7) | 0.479‡ | 32 (10.7) | 45 (10.2) | 0.812‡ |
| Work-related COVID-19 exposure in emergency department, n (%) | 1 (2.9) | 12 (4.9) | 1.000§ | 20 (7.5) | 14 (7.1) | 0.858‡ | 21 (7.0) | 26 (5.9) | 0.527‡ |
| Work-related COVID-19 exposure in other departments, n (%) | 1 (2.9) | 8 (3.3) | 1.000§ | 13 (6.6) | 7 (2.6) | 0.039‡ | 8 (2.7) | 21 (4.7) | 0.155‡ |
| Any exposure to secretions from infected patients, n (%)* | 3 (11.1) | 44 (19.6) | 0.287‡ | 57 (28.9) | 45 (28.0) | 0.837‡ | 60 (26.8) | 89 (23.1) | 0.301‡ |
| COVID-19 history in colleagues, n (%) | 22 (64.7) | 150 (61.0) | 0.675‡ | 239 (90.2) | 179 (90.9) | 0.807‡ | 261 (87.3) | 329 (74.3) | <0.001‡ |
| Use of any PPE, n (%) | 34 (100.0) | 242 (98.4) | 1.000§ | 257 (97.0) | 184 (93.4) | 0.068‡ | 291 (97.3) | 426 (96.2) | 0.390‡ |
| Use of disposable gloves, n (%) | 30 (88.2) | 193 (78.5) | 0.184‡ | 186 (70.2) | 126 (64.0) | 0.157‡ | 216 (72.2) | 319 (72.0) | 0.945‡ |
| Use of gowns, n (%) | 30 (88.2) | 201 (81.7) | 0.348‡ | 188 (70.9) | 122 (61.9) | 0.041‡ | 218 (72.9) | 323 (72.9) | 0.999‡ |
| Use of aprons, n (%) | 14 (41.2) | 79 (32.1) | 0.293‡ | 46 (17.4) | 45 (22.8) | 0.143‡ | 60 (20.1) | 124 (28.0) | 0.014‡ |
| Use of surgical masks, n (%) | 29 (85.3) | 221 (89.8) | 0.384§ | 228 (86.0) | 173 (87.8) | 0.576‡ | 257 (86.0) | 394 (88.9) | 0.224‡ |
| Use of respirators (N95/FFP2/FFP3), n (%) | 29 (85.3) | 186 (75.6) | 0.210‡ | 200 (75.5) | 137 (69.5) | 0.156‡ | 229 (76.6) | 323 (72.9) | 0.260‡ |
| Use of facial protectors, n (%) | 23 (67.6) | 177 (72.0) | 0.603‡ | 166 (62.6) | 101 (51.3) | 0.014‡ | 189 (63.2) | 278 (62.8) | 0.899‡ |
| Use of goggles, n (%) | 16 (47.1) | 139 (56.5) | 0.299‡ | 88 (33.2) | 62 (31.5) | 0.694‡ | 104 (34.8) | 201 (45.4) | 0.004‡' |

Bold indicates statistical significance, and the missing p-value is due to the low number of cases. *The missing values were due to unanswered online survey questions for age in 26, occupation in one, the exposure to secretions from infected patients in 132 responses. The item for any household member older than 60 years of age was only replied by the participants who reported that they did not live single. †Mann–Whitney U test. ‡Pearson's $\chi^2$ test. §Fisher's exact test. COPD: chronic obstructive pulmonary disease; COVID-19: coronavirus disease 2019; HCW: health care worker; min–max: minimum–maximum; PPE: personal protective equipment.

Table 2. Crude and age- and sex-adjusted logistic regression analysis of the association between selected parameters and COVID-19 in health care workers.

| | Crude analysis | | | Adjusted analysis | |
|---|---|---|---|---|---|
| | n | OR (95%CI) | p-value | OR (95%CI) | p-value |
| Smoking status | 716 | | | | |
| Never smoker | | 1.00 (ref) | | 1.00 (ref) | |
| Ex-smoker | | 0.99 (0.67–1.45) | 0.947 | 1.04 (0.69–1.55) | 0.868 |
| Current smoker | | 0.38 (0.23–0.63) | <0.001 | 0.38 (0.23–0.63) | <0.001 |
| Asthma | 716 | 1.62 (0.98–2.69) | 0.060 | 1.66 (1.00–2.75) | 0.050 |
| Working at the onset of the outbreak | 716 | 3.88 (1.13–13.37) | 0.032 | 3.76 (1.09–12.98) | 0.036 |
| Any work-related COVID-19 exposure | 716 | 0.84 (0.62–1.15) | 0.276 | 0.84 (0.62–1.14) | 0.258 |
| Any exposure to secretions from infected patients | 588 | 1.14 (0.77–1.68) | 0.522 | 1.11 (0.75–1.65) | 0.607 |
| Not working at the time of survey | 716 | 5.67 (3.34–9.64) | <0.001 | 5.69 (3.35–9.67) | <0.001 |
| COVID-19 history in colleagues | 716 | 2.29 (1.52–3.44) | <0.001 | 2.27 (1.51–3.41) | <0.001 |
| Non-work-related COVID-19 exposure | 716 | 4.66 (2.72–7.99) | <0.001 | 4.72 (2.74–8.14) | <0.001 |
| COVID-19 exposure at home | 716 | 6.45 (3.50–11.90) | <0.001 | 6.52 (3.52–12.08) | <0.001 |
| COVID-19 history in any household member | 716 | 7.98 (5.42–11.74) | <0.001 | 8.16 (5.52–12.08) | <0.001 |
| Use of disposable gloves | 716 | 1.03 (0.74–1.44) | 0.853 | 1.00 (0.71–1.40) | 0.976 |
| Use of gowns | 716 | 0.96 (0.69–1.35) | 0.823 | 0.96 (0.68–1.34) | 0.796 |
| Use of aprons | 716 | 0.64 (0.45–0.92) | 0.017 | 0.62 (0.43–0.90) | 0.011 |
| Use of surgical masks | 716 | 0.80 (0.51–1.26) | 0.340 | 0.80 (0.51–1.27) | 0.341 |
| Use of respirators (N95/FFP2/FFP3) | 716 | 1.19 (0.84–1.68) | 0.323 | 1.17 (0.82–1.65) | 0.388 |
| Use of facial protectors | 716 | 1.07 (0.78–1.46) | 0.676 | 1.06 (0.78–1.44) | 0.725 |
| Use of goggles | 716 | 0.64 (0.47–0.88) | 0.005 | 0.64 (0.47–0.87) | 0.005 |

CI: confidence interval; COVID-19: coronavirus disease 2019; HCW: health care worker; OR: odds ratio; ref: reference. Bold indicates statistical significance.

align with the COVID-19 in HCWs. The study also observed an inverse relationship between the use of aprons and goggles and COVID-19 in HCWs. Even in the early days of the pandemic, occupational risk factors in HCWs regarding COVID-19 were documented. According to a rapid review, the hospital division where the HCW worked and the use of PPE, particularly masks, were the parameters found related to COVID-19 in HCWs[10]. In an evaluation of 4,664 Swiss HCWs, Kahlert et al.[11] showed that close contact with patients with COVID-19 and exposure to co-workers with COVID-19 were related to COVID-19 in HCWs. This study also evaluated the types of PPE used during close contact and revealed an inverse relationship between the use of any face mask, gloves, gown, and goggles and COVID-19, but a direct relationship with nonusage of PPE. Our results are compatible with the similar studies in the literature.

We observed that the variables associated with COVID-19 in HCWs, other than occupational characteristics, included non–work-related COVID-19 exposure, COVID-19 exposure at home, and a COVID-19 history among household members. Several studies also evaluated nonoccupational factors in HCWs. Kahlert et al.[11] revealed that a COVID-19–positive household member and a history of visiting a COVID-19 hotspot were related to COVID-19 in HCWs. Çelebi et al.[12] demonstrated that having a SARS-CoV-2–positive household member bore a significant relationship to COVID-19 in HCWs. Combining these results, we consider that nonoccupational risk factors are also integral for COVID-19 in HCWs depending on the increased community transmission during the outbreak.

The results showed a statistically significant difference in smoking status between HCWs who contracted COVID-19 and those without a COVID-19 history. Moreover, current smoking is inversely related to COVID-19 in HCWs (OR=0.38, 95% CI: 0.23–0.63) when nonsmoking respondents were accepted as the reference. Since the beginning of the pandemic, the association between smoking status and COVID-19 has been investigated, and alternative biological mechanisms have been proposed to suggest an increased or decreased risk for COVID-19 due to

smoking[13]. The number of studies and meta-analyses documenting the relationship between smoking and the severity of COVID-19 has increased[14]. However, Kahlert et al.[11] showed a similar result to this study for active smoking. More substantial prospective studies are required to document if the risk of contracting COVID-19 changes according to the smoking status and relevant mechanisms.

According to the results, 59.2% of HCW who contracted COVID-19 were asymptomatic. A meta-analysis estimated that 40% of RT-PCR positive HCWs were asymptomatic[15]. The results also showed that the most prevalent symptoms were cough, headache, and loss of smell and/or taste. Similarly, an observational study found the prevalence of cough as 82.2% in 185 symptomatic and COVID-19–positive Belgian HCWs[16]. Despite varying frequencies according to the study design, these results indicate the need for a screening program for both symptomatic and asymptomatic HCWs regarding the risk status.

The strengths of this study include representation of the national profile due to a wide range of participants from different provinces of Turkey, more varied items investigating both occupational and nonoccupational parameters in HCWs in terms of contracting COVID-19, and a two-phase design to evaluate temporal change over time. However, the study has some limitations. Online surveys have classical constraints about the percentage of participation, the representativeness of the sample of the wider population, and data collection and quality, despite a relatively longer duration for the data collection being applied in both phases. The nature of the data collection method may favor the participation of HCWs with a history of the nonsevere disease, although the survey questions did not address the severity of COVID-19 in HCWs. The cumulative probability of exposing occupational and nonoccupational risks during the pandemic increases; however, most survey questions for occupational and nonoccupational parameters did not include a temporal and quantitative evaluation. This strategy might have caused a limitation in the grading of the risks.

## CONCLUSIONS

Occupational and nonoccupational parameters are related to COVID-19 in HCWs. Active surveillance, including the diagnosis of both symptomatic and asymptomatic HCWs, and documenting and controlling occupational and nonoccupational risks should be maintained. Future prospective studies may document the changes related to dynamic features of an ongoing pandemic.

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## AUTHORS' CONTRIBUTIONS

**AS:** Conceptualization, Data curation, Formal Analysis, Investigation, Methodology, Project administration, Supervision, Visualization, Writing – original draft. **ZNT:** Data curation, Formal Analysis, Methodology, Writing – original draft. **CS:** Conceptualization, Data curation, Methodology, Supervision, Writing – review & editing. **PMA:** Conceptualization, Formal Analysis, Investigation, Methodology, Project administration, Supervision, Writing – review & editing.

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# Comparison of samples found positive by anti-HCV screening test with line immunoassay and determination of threshold value

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## SUMMARY

**OBJECTIVE:** This study aimed to compare the serum samples found reactive (≥1–≤20 signal-to-cutoff ratio) with Elecsys antibodies to hepatitis C virus screening test with innogenetics-line immunassay hepatitis C Virus Score test and to determine the most appropriate threshold value for our country, since positive results close to the cutoff value cause serious problems in routine diagnostic laboratories.

**METHODS:** Antibodies to hepatitis C virus-positive samples from 687 different patients were included in the study. Antibodies to hepatitis C virus antibody detection was performed using Elecsys antibodies to hepatitis C virus II kits (Roche Diagnostics, Germany), an electrochemiluminescence method based on the double-antigen sandwich principle, on the Cobas e601 analyzer (Roche Diagnostics) in accordance with the recommendations of the manufacturer. Samples that were initially identified as reactive were studied again. Samples with ≥1–≤20 signal-to-cutoff ratio reagents as a result of retest were included in the study to be validated with the third-Generation Line immunassay kit (innogenetics-line immunassay hepatitis C Virus, Belgium).

**RESULTS:** A total of 687 samples with antibodies to hepatitis C virus positive and levels between 1–20 S/Co were found to be 56.1% negative, 14.8% indeterminate, and 29.1% positive by innogenetics-line immunassay hepatitis C Virus confirmation test. When the cases with indeterminate innogenetics-line immunassay hepatitis C Virus test results were accepted as positive, the signal-to-cutoff ratio value for antibodies to hepatitis C virus was determined as 5.8 (95% confidence interval) in distinguishing the innogenetics-line immunassay hepatitis C Virus negative and positive groups.

**CONCLUSION:** It was concluded that with further studies on this subject, each country should determine the most appropriate S/Co value for its population, and thus it would be beneficial to reduce the problems such as test repetition and cost increase.

KEYWORDS: Hepatitis C virus antibodies. Enzyme immunoassay. Hepatitis C virus. Immunoassay.

## INTRODUCTION

Early detection of hepatitis C virus (HCV) antibodies is the first step in the management of chronic hepatitis and identification of patients who are in need of treatment[1,2]. First-generation anti-HCV tests were developed in 1990 using the recombinant c100-3 epitope of the NS4 protein and have limited sensitivity and specificity[1,3]. A second-generation assay was soon developed using a multi-antigen format, including epitopes from the core, NS3 and NS4 proteins[1,4]. In the early 2000s, third-generation tests were introduced to detect the presence of antibodies against recombinant core, NS3, NS4, and NS5 antigens of the virus. The test format had also changed from an enzyme immunoassay (EIA) method to a chemiluminescent immunoassay (CLIA) method, with a marked improvement in performance[1,5]. The Elecsys anti-HCV II test (Roche Diagnostics, Germany) works with the

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electrochemiluminescence (ECLIA) method and is based on the double-antigen sandwich principle[5]. The INNO-LIA HCV score test (Innogenetics, Belgium) is a line immunoassay (LIA) method recommended for use as an additional test for samples found reactive in the anti-HCV screening procedure. The INNO-LIA HCV Score assay uses well-defined antigens derived from HCV immunodominant proteins from the core, the E2 hypervariable region (HVR), the NS3 helix region, and the NS4A, NS4B, and NS5A regions[6].

In societies with low prevalence of HCV, such as our country, and in those with autoimmune disease, it has been reported that tests used to detect anti-HCV antibodies give high false-positive results[7]. Positive results close to the cutoff value cause serious problems in routine diagnostic laboratories (such as reporting problems, increased costs due to repeat testing or HCV-RNA testing). In contrast, the presence of HCV-RNA often does not accompany close to the cutoff value or low anti-HCV positivity[8]. In this study, it was aimed to compare the samples with positive results (≥1–≤20 S/Co) with the Elecsys anti-HCV screening test with the INNO-LIA Score test and to determine the threshold value for the anti-HCV screening test.

# METHODS

A total of 687 anti-HCV–positive (≥1–≤20 S/Co) serum samples from different patients sent to Erciyes University Medical Faculty Central Laboratory Serology Unit between January 2018 and April 2021 were included in the study. Then, the same sera were studied with the Third-Generation LIA kit (INNO-LIA, HCV Score, Innogenetics, Belgium). The results of both tests were evaluated retrospectively.

## Anti-HCV Antibody Detection

Anti-HCV antibody detection was performed using Elecsys anti-HCV II kits (Roche Diagnostics), an ECLIA method based on the double-antigen sandwich principle, on the Cobas e601 analyzer (Roche Diagnostics) in accordance with the recommendations of the manufacturer. Samples with <1 S/Co value were considered nonreactive, while samples with ≥1 S/Co value were considered reactive. Samples that were initially identified as reactive were studied again. Samples with ≥1 and ≤20 S/Co reagents as a result of retest were included in the study to be validated with the Third-Generation LIA kit.

## Line Immunoassay

Anti-HCV–positive samples were studied with the Third-Generation INNO-LIA HCV score test (Innogenetics, Belgium) kits containing the C1, C2, E2, NS3, NS4, and NS5 regions of the HCV genome in accordance with the recommendations of the manufacturer and were interpreted as negative, indeterminate, and positive.

Negative: All HCV antigen bands have a negative reactivity degree or one of the HCV antigen bands, except that NS3 has ± reactivity.

Positive: Reactivity of ± or higher in at least two HCV antigen bands.

Indeterminate: Any HCV antigen line has a reactivity rating of 1+or higher, or the NS3 band has reactivity of±or more, while all other antigen lines are negative.

## Statistical analysis

Data were evaluated using statistical package program IBM SPSS Statistics for Windows, version 26.0 (IBM Corp. Released 2019, Armonk, NY, USA). In comparisons according to the INNO-LIA HCV score verification test result categories, which had more than two subcategories, the Elecsys anti-HCV II screening test continuous measurement value distribution was evaluated by the Kruskal–Wallis test based on the normality test result. The Bonferroni test was used as a multiple comparison test. A $p<0.05$ value was considered statistically significant. In addition, the analysis of the data was performed with the MedCalc 15.8 program. As a result of the application of the INNO-LIA HCV score confirmation test, the Elecsys anti-HCV II screening test results of patients with anti-HCV positive; receiver operating charateristic (ROC) curve analysis was applied to determine the cutoff point as negative group with (indeterminate+positive) group and positive group with (negative+indeterminate) group.

# RESULTS

A total of 687 samples were anti-HCV positive with results ranging from 1–20 S/Co, 385 (56.1%) were negative with the INNO-LIA HCV score confirmatory test, 102 (14.8%) were indeterminate, and 200 (29.1%) was found positive. It was found that the distribution of anti-HCV–positive values by categories as a result of the INNO-LIA HCV score verification test did not provide the normal distribution assumption. The descriptive statistics and median value and the 25th and 75th percentile values of continuous measurement values that are anti-HCV positive and whose value varies between 1–20 S/Co according to each INNO-LIA HCV score test result categories are given in Table 1. The distribution of the measurement values of the anti-HCV–positive group according to the Kruskal–Wallis test result shows statistically significant differences according to the INNO-LIA HCV score result categories ($p<0.001$).

ROC curve analysis was used to determine the cutoff point of the negative group with (indeterminate+positive) group and the positive group with (indeterminate+negative) group as a result of

application of the INNO-LIA HCV score confirmation test to the results found positive with the Roche Elecsys anti-HCV II test (Figures 1 and 2). As a result of the analysis, when the cases that were indeterminate according to the INNO-LIA HCV score test result were considered positive, the S/Co value for anti-HCV was determined as 5.8 (95% confidence interval) in distinguishing the INNO-LIA HCV score test negative and positive groups. At S/Co values >5.8, sensitivity was 79.1%, specificity 78.2%, positive predictive value 73.8%, and negative predictive value 82.6%. When the cases that were indeterminate according to the INNO-LIA HCV score test result were considered negative, the S/Co value for anti-HCV was determined as 7.3 (95% confidence interval) for the INNO-LIA HCV score test in distinguishing the negative and positive groups. At S/Co values >7.3, the sensitivity was 81%, the specificity was 79.1%, the positive predictive value was 61.1%, and the negative predictive value was 91%.

## DISCUSSION

The diagnosis of HCV infection usually begins with the detection of anti-HCV using EIA and a CLIA screening methods[9]. Direct HCV-RNA testing is recommended in anti-HCV-positive patients with clinically acute or chronic liver disease due to the possibility of false-positive results in populations where prevalence is low[10]. However, high costs, labor-intensive procedures, and the need for specialized equipment and qualified personnel limit the widespread use of molecular techniques[9,11]. Furthermore, deciding on a reliable, easy-to-use, and cost-effective test to predict true HCV infection status or HCV viremia in anti-HCV reactive patients remains controversial. Although it is recommended to confirm with tests such as Recombinant Immunoblot Assay (RIBA) when a low S/Co result is obtained in the classical diagnosis algorithm of HCV, these tests are likely to yield "indeterminate" results[12]. The Centers for Disease Control and Prevention (CDC) removed the RIBA test from the new algorithm and explained that the cutoff value of ≥1 S/Co should be adjusted according to the characteristics of the population[8]. In addition, the CDC has proposed predictive cutoff values for some commercially available anti-HCV screening tests. For example, Architect (Abbott Laboratories, USA) has set a threshold value of ≥5 S/Co for the anti-HCV screening test, but these values have not yet been specified for the Roche Elecsys anti-HCV II tests[9,13]. Lai et al.[14] reported that when the S/Co ratio is <3.0 or ≥20.0, there is no need for anti-HCV confirmatory testing with RIBA because of the high true negative and high true positive rate, respectively. They also reported that the RIBA confirmatory test is required for patients with an S/Co ratio of 3.0–19.9, due to possible false-positive results given by the ECLIA. Results between 1.0 and 20 S/Co with the Elecsys anti-HCV II screening test in our laboratory are confirmed by the INNO-LIA HCV score test.

In our study, a total of 687 samples with reactive anti-HCV results (1–20 S/Co) were tested with the INNO-LIA HCV score confirmation test. It was found that 56.1% were negative,

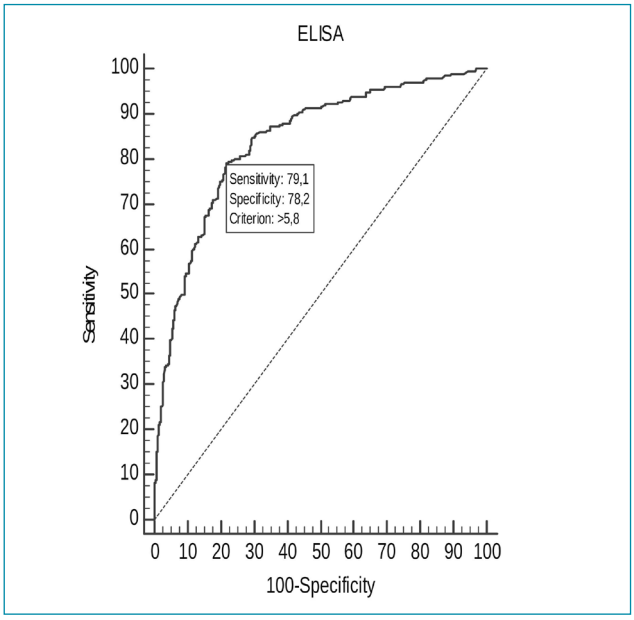


Figure 1. Receiver operating charateristic curve analysis of negative group with (indeterminate+positive) group.

Table 1. Evaluation of the distribution of Elecsys Antibodies to hepatitis C virus II screening test measurement values of patients with Antibodies to hepatitis C virus positive according to the confirmatory test Innogenetics- line immunassay hepatitis C virus score test findings.

| INNO-LIA HCV score | n (%) | $\overline{X} \pm SS$<br>M ($Q_1$–$Q_3$) | p-value⁺ | Pairwise comparisons |
|---|---|---|---|---|
| Negative | 385 (56.0) | 4.21 ± 3.74<br>2.66 (1.54–5.40) | $\chi^2$=274.140;<br>p<0.001 | 1–2: p<0.001 |
| Positive | 200 (29.2) | 12.59 ± 5.20<br>12.86 (8.36–16.83) | | 1–3: p<0.001 |
| Indeterminate | 102 (14.8) | 7.29 ± 4.56<br>6.53 (3.63–10.00) | | 2–3: p<0.001 |

⁺Kruskal Wallis Test. INNO-LIA HCV score: innogenetics-line immunassay hepatitis C virus

14.8% were indeterminate, and 29.1% were positive. It has been reported that a total of 47041 samples, which were found to be anti-HCV reactive by EIA method, was found to be positive in 49.3%, indeterminate in 17.1%, and negative in 33.5% by RIBA method[15]. In another study, two different anti-HCV systems (Cobas e411 Elecsys anti-HCV II and Vidas anti-HCV Biomerieux) were compared with the INNO-LIA HCV score test using 1931 serum samples. It has been reported that the performance agreement of Vidas and INNO-LIA for discrepant samples is 65%, and the percentage agreement is 80% for Vidas-negative samples and 28% for Vidas-positive samples. It was stated that Cobas had a performance agreement of 41% with INNO-LIA in discrepant samples, and the percentage agreement was 28% for Cobas negative samples and 72% for Cobas positive samples[16]. In a study where Architect i2000SR (Abbot Laboratories) and Vidas systems were used as anti-HCV screening test, 70 serum samples with low positive (1≤ S/Co <8) were compared with the INNO-LIA HCV score assay. It has been reported that the agreement between the Architect i2000SR and the INNO-LIA HCV score assay is 42.6%, and the percentage agreement between Vidas and the INNO-LIA HCV score assay is 79.4% [17]. A multicenter study conducted in Turkey reported that 67% of 10050 anti-HCV–positive serum samples were positive with RIBA[18]. In another study, this rate was found to be 61.4%[8]. In our study, when the indeterminate results were considered positive, this rate was found to be 49.5%.

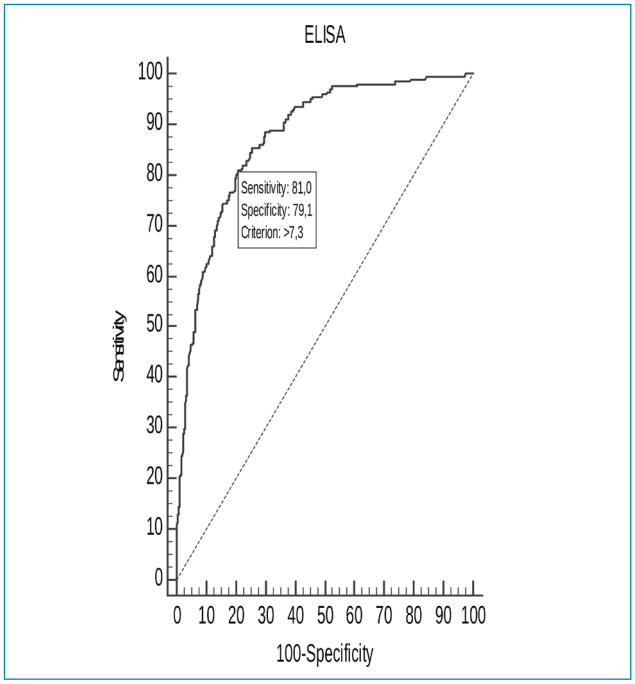


Figure 2. Receiver operating charateristic curve curve analysis of positive group with (indeterminate+negative) group.

Yang et al.[1] reported that in the Elecsys anti-HCV II assay, an S/Co ratio of 20.0 predicted a true positive result ≥95% of the time. On the other hand, Wu et al.[19] found that this value was 12.0 for the InTec test (InTec products, China) and 5.0 for the Architect test in their study in which they investigated the appropriate S/Co thresholds. Saribas et al.[8] determined the S/Co value of 7.2 (95% confidence interval) for Architect anti-HCV in distinguishing LIA positive and negative groups when LIA indeterminate cases were considered negative. In our study, when the results found indeterminate by the INNO-LIA HCV score assay were considered positive and negative, the S/Co ratios for anti-HCV were found to be 5.8 and 7.3, respectively.

## CONCLUSIONS

The lack of HCV-RNA results in each patient limited us to make a comparison in this respect. Although the INNO-LIA HCV score assay is used as a complementary test in the detection of anti-HCV antibodies, it has the disadvantage of visual evaluation and highly uncertain results. As a result, it is necessary for each country to determine the most appropriate S/Co value for its population, with more studies to be done on this subject. This will reduce patient victimization due to reporting problems and problems such as increased cost due to repeated testing or the need for HCV-RNA testing.

## AUTHORS' CONTRIBUTIONS

**MAA:** Conceptualization, Investigation, Writing – review & editing. **PS:** Conceptualization, Data curation, Writing – review & editing. **MO:** Data curation, Writing – original draft, Writing – review & editing. **BE:** Formal analysis.

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# Factors associated with postoperative complications following appendectomy in elderly patients

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## SUMMARY

**OBJECTIVE:** Appendicitis in elderly patients is more challenging due to delayed presentation and higher comorbidities, which are associated with increased postoperative morbidity. The aim of this study was to evaluate factors that predict 30-day complications in elderly patients undergoing appendectomy.

**METHODS:** The records of elderly patients who underwent appendectomy were reviewed. The primary outcome was 30-day postoperative complications. Independent variables examined included demographic data, comorbidities, preoperative laboratory values, pathological findings, and surgical features. Both univariate and multivariate regression analyses were performed to identify factors associated with postoperative complications.

**RESULTS:** Evaluation was performed on 80 patients, comprising 63.8% females with a mean age of 71.3 years. Notably, 19 (23.8%) patients had one or more complications within 30 days after surgery. No significant difference was found between patients with and without complications in respect of age, gender, or laboratory features. The rates of American Society of Anesthesiologists scores 3–4 (p=0.006), hypertension (p=0.016), cardiovascular disease (p=0.049), and obesity (p=0.040) were significantly higher for patients with complications than for those without. On multivariate analysis, obesity (OR 9.41), chronic obstructive pulmonary disease (OR 9.72), and open appendectomy (OR 14.87) were independently associated with 30-day postoperative complications.

**CONCLUSIONS:** Older patients undergoing appendectomy tend to have poorer outcomes than younger patients. Therefore, it is critical to identify factors that could reduce the possibility of adverse outcomes in this frail population. The results of this study suggest that obesity, chronic obstructive pulmonary disease, and an open approach are independent factors for complications in elderly patients undergoing appendectomy.

KEYWORDS: Appendicitis. Appendectomy. Elderly. Postoperative complications.

## INTRODUCTION

Acute appendicitis is a common surgical emergency worldwide, which has a lifetime risk of approximately 7%[1]. Although more frequently diagnosed in younger patients, 5–10% of cases occur over the age of 60 years[2]. An aging population driven by a global increase in life expectancy is likely to result in an increased incidence of acute appendicitis in elderly individuals over time[3,4]. Older patients undergoing appendectomy tend to have poorer outcomes than their younger counterparts[3]. The rates of perioperative morbidity and mortality are higher in elderly patients who are more likely to have concomitant comorbidities and often have delayed presentation and atypical symptoms, leading to an increased risk of complicated appendicitis, appendiceal perforation, intra-abdominal abscess, and even mortality[5,6]. Reported complication rates following appendectomy in the elderly range from 10–40%, with mortality rates as high as 3%[2,5,7-10]. Therefore, understanding and identifying risk factors that predict postoperative outcomes for elderly patients undergoing appendectomy are important steps in managing these patients. The aim of this study was to

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evaluate factors that might be predictive of 30-day complications following appendectomy in elderly patients.

# METHODS

## Study design and patients

The medical records were retrospectively reviewed of elderly patients (aged ≥65 years) who underwent appendectomy for acute appendicitis at a tertiary level hospital in Turkey between January 2017 and December 2020. Patients who underwent an appendectomy as part of another major operation or those with appendiceal malignancy were excluded from the study. This study was approved by the Ethics Committee of Gulhane Training and Research Hospital (2021-51) and was registered at Clinicaltrials.gov (NCT04791657).

## Data collection

Data collected included age, sex, body mass index (BMI), American Society of Anesthesiologists (ASA) classification, comorbidities, preoperative laboratory values, diagnostic studies, surgical techniques and surgical procedures, intensive care unit (ICU) stay, length of hospital stay, in-hospital mortality, and 30-day outcomes. The diagnosis of appendicitis was confirmed with histological evaluation. Complicated appendicitis is defined as cases with peri-appendicular abscess, gangrenous appendicitis, and/or perforation of the appendix noted on radiological imaging studies, operative notes, or pathological results.

## Outcome variables

The primary clinical outcome of interest was 30-day postoperative complications. The Clavien–Dindo classification was used to grade postoperative outcomes and complications[11]. Secondary outcomes were the rate of complicated appendicitis, in-hospital mortality, and 30-day readmission, which was defined as an unplanned readmission to hospital within 30 days of discharge.

## Statistical analysis

Examinations of normal distribution assumptions for continuous variables were visually assessed with quantile–quantile plots and histograms and confirmed with the Shapiro–Wilk test. Categorical data were presented as number (n) and percentage (%), and continuous data as mean±standard deviation or median with range values, depending on the distribution assumptions. Associations between variables were evaluated using the Student's *t*-test or the Mann-Whitney U test (for continuous variables) and the Pearson's $\chi^2$ or Fisher's exact test (for categorical variables), where appropriate. Factors identified at $p<0.20$ in univariate analysis were selected for inclusion in a multivariate logistic regression model to assess the independent effect of these variables in the presence or absence of 30-day postoperative complications. Odds ratios (ORs) with 95% confidence intervals (CIs) were calculated. All tests were two-sided, and a $p<0.05$ was considered statistically significant. All statistical analyses were performed in RStudio statistical software, version 1.3.1093 (RStudio, Inc., Boston, MA, USA). A forest plot was created according to the result of the multivariate logistic analysis, using GraphPad Prism version 8.0.1 for Windows (GraphPad Software Inc., CA, USA).

# RESULTS

The study population consisted of 80 patients, and 51 (63.8%) patients were female with a mean age of 71.3±5.9 years. Overall, 28.8% of patients had no comorbidity, and 71.2% had at least one comorbid disease.

Preoperative ultrasonography was performed in all patients and computed tomography was performed in 53 (66.3%) patients. In respect of surgical technique, an open approach was applied to 58 (72.5%) cases and the laparoscopic technique to 22 (27.5%). A total of 31 (38.8%) patients had complicated appendicitis, and 49 (61.3%) patients were uncomplicated. ICU admission after the surgical treatment of appendicitis was required by 28.8% of all the patients. The mean ICU stay was 0.7 days, and the average hospital stay was 6 days. The in-hospital mortality rate was 1.3% (n=1), and 30-day readmission and 30-day rehospitalization rates were 12.5 and 2.5%, respectively. The demographic characteristics and perioperative data of the patients are presented in Table 1.

Overall, 19 (23.8%) patients had one or more complications after surgery. Of these patients, 8 (42.1%) developed wound infection. Other complications included wound dehiscence in two patients, wound seroma or hematoma in two patients, prolonged ileus in three patients, bowel obstruction in one patient, atelectasis in one patient, and respiratory infection in one patient. One death was due to sepsis secondary to intestinal obstruction and evisceration.

The comparative analysis of patients with 30-day complications and those without complications is summarized in Table 2. No statistically significant difference was found between patients with and without complications in respect of age, gender, or laboratory features including leukocyte and C-reactive protein values. Patients with complications had a significantly higher prevalence of ASA score 3 or 4 (52.6 *versus* 18.0%, p=0.006), obesity (BMI ≥30 kg/m$^2$) (47.4 *versus* 23.0%, p=0.040), hypertension (78.9 *versus* 47.5%, p=0.016), and cardiovascular disease (CVD) (42.1 *versus* 19.7%, p=0.049) than those without

Table 1. Demographic characteristics, preoperative evaluation, clinicopathological features, and postoperative outcomes of the study population (n=80).

| Demographic characteristics | |
|---|---|
| Age (years) | 71.3±5.9 (range, 65–87) |
| Female gender, n (%) | 51 (63.8) |
| Body mass index (kg/m$^2$) | 28.1±5.9 (range, 15.7–47.8) |
| Comorbidities | |
| ASA classification, n (%) | |
| 1–2 | 59 (73.8) |
| 3–4 | 21 (26.3) |
| Hypertension, n (%) | 44 (55.0) |
| Diabetes mellitus, n (%) | 23 (28.8) |
| Cardiovascular disease, n (%) | 20 (25.0) |
| COPD, n (%) | 8 (10.0) |
| Kidney disease, n (%) | 5 (6.3) |
| Preoperative evaluation | |
| Leukocyte (×10$^9$/L) | 14.2±5.0 (range, 6.0–28.1) |
| Neutrophil (×10$^9$/L) | 11.4±4.9 (range, 1.5–24.5) |
| Lymphocyte (×10$^9$/L) | 1.6±0.7 (range, 0.5–3.7) |
| C-reactive protein (mg/L) | 147.7±109.3 (range, 1.5–457) |
| Diagnostic study, n (%) | |
| Only USG | 27 (33.8) |
| USG and CT | 53 (66.3) |
| Surgical and pathological features | |
| Surgical technique, n (%) | |
| Open | 58 (72.5) |
| Laparoscopic | 22 (27.5) |
| Pathological findings, n (%) | |
| Uncomplicated appendicitis | 49 (61.3) |
| Complicated appendicitis | 31 (38.8) |
| Postoperative outcomes | |
| ICU stay, n (%) | 23 (28.8) |
| Length of ICU stay (days) | 0.7±1.9 (0–14) |
| Length of hospital stay (days) | 6.0±4.7 (range, 1–24) |
| In-hospital mortality, n (%) | 1 (1.3) |
| 30-Day complication, n (%) | 19 (23.8) |
| 30-Day readmission, n (%) | 10 (12.5) |
| 30-Day rehospitalization, n (%) | 2 (2.5) |

ASA: American Society of Anesthesiologists; COPD: chronic obstructive pulmonary disease; CT: computed tomography; ICU: intensive care unit; USG: ultrasonography.

complications. The rate of complicated appendicitis did not differ between patients with and without 30-day complications (31.6 *versus* 41.0%, p=0.462). The length of ICU stay, hospital stay, and the 30-day readmission rate were higher in patients with complications (p=0.018, p<0.001, and p=0.001; respectively), and the 30-day rehospitalization rate was similar in both groups (p=0.421).

Univariate and multivariate logistic regression analyses were performed to determine the factors that predicted 30-day complications. Univariate regression analysis showed that age, male gender, obesity, ASA scores 3–4, hypertension, CVD, COPD, and open approach were associated with an increased risk of developing postoperative complications. In the multivariate analysis, only obesity (OR 9.41, 95%CI 1.79–14.31), COPD (OR 9.72, 95%CI 1.21–77.78), and open appendectomy (OR 14.87, 95%CI 1.55–142.33) were found to be independently associated with increased 30-day postoperative complications (Figure 1).

## DISCUSSION

Despite a significant decrease in morbidity and mortality rates after the surgical treatment of acute appendicitis in elderly patients over the past five decades, nearly one-third of older patients undergoing appendectomy still have postoperative complications[2,7,8,10,12]. When considering the increase in life expectancy and aging population in the coming years, the number of elderly patients with appendicitis appears to be increasing[1-3]. This will most likely lead to more patients with complications and comorbid conditions, potentially leading to increased resource utilization and increased economic cost for health care systems[8,13,14]. However, a limited number of studies have been conducted to evaluate the predictors of postoperative outcomes in elderly individuals undergoing emergency appendectomy[2,8,9,12]. Therefore, this study aimed to identify the risk factors that predict postoperative complications in elderly patients undergoing appendectomy.

In the analysis of 80 elderly patients with a mean age of 71.3 years in this study, nearly three quarters had at least one comorbid condition, and 30-day postoperative complications developed in 23.8% of the patients, which was similar to the findings of the previous studies that have reported rates ranging from 10% to 40%. The most common complications were superficial wound infection, prolonged ileus, deep wound infection, wound dehiscence, and wound seroma/hematoma. Patients with complications within postoperative 30 days had a significantly higher prevalence of ASA score 3 or 4, obesity, hypertension, and CVD than those without complications. The length of ICU stay, hospital stay, and the 30-day readmission rate were the other

Table 2. Comparison of the patients with postoperative complications and those without complications

| | Patients without complications (n=61) | Patients with complications (n=19) | p-value |
|---|---|---|---|
| Age (years)* | 69 (65–87) | 74 (65–84) | 0.088 |
| Male gender, n (%) | 19 (31.1) | 10 (52.6) | 0.089 |
| Body mass index (kg/m$^2$), n (%) | | | **0.040** |
| <30 | 47 (77.0) | 10 (52.6) | |
| ≥30 | 14 (23.0) | 9 (47.4) | |
| ASA classification, n (%) | | | **0.006** |
| 1–2 | 50 (82.0) | 9 (47.4) | |
| 3–4 | 11 (18.0) | 10 (52.6) | |
| Hypertension, n (%) | 29 (47.5) | 15 (78.9) | **0.016** |
| Diabetes mellitus, n (%) | 17 (27.9) | 6 (31.6) | 0.755 |
| Cardiovascular disease, n (%) | 12 (19.7) | 8 (42.1) | **0.049** |
| COPD, n (%) | 4 (6.6) | 4 (21.1) | 0.086 |
| Kidney disease, n (%) | 4 (6.6) | 1 (5.3) | 1.000 |
| Leukocyte (×10$^9$/L)† | 14.1±5.0 | 14.3±5.0 | 0.879 |
| C-reactive protein (mg/L)* | 120 (1.5–409) | 142 (34–457) | 0.263 |
| Surgical technique, n (%) | | | 0.058 |
| Open | 41 (65.6) | 17 (89.5) | |
| Laparoscopic | 20 (32.8) | 2 (10.5) | |
| Complicated appendicitis, n (%) | 25 (41.0) | 6 (31.6) | 0.462 |
| ICU stay, n (%) | 14 (23.0) | 9 (47.4) | **0.040** |
| Length of ICU stay (days)* | 0 (0–6) | 0 (0–14) | **0.018** |
| Length of hospital stay (days)* | 4 (1–14) | 5 (1–24) | **<0.001** |
| In-hospital mortality, n (%) | 0 | 1 (5.3) | 0.237 |
| 30-Day readmission, n (%) | 3 (4.9) | 7 (36.8) | **0.001** |
| **30-Day rehospitalization, n (%)** | **1 (1.6)** | **1 (5.3)** | **0.421** |

ASA: American Society of Anesthesiologists; COPD: chronic obstructive pulmonary disease; ICU: intensive care unit stay. *Values are represented as median (range). †Data are expressed as mean±standard deviation. Statistically significant results (p<0.05) were written in bold format

statistically significant factors that were higher in patients with complications. However, these factors were not included in the regression model, because they reflect an association rather than a cause-and-effect relationship; it might be possible that early postoperative complications cause longer ICU and hospital stay. The results of the multivariate logistic regression analysis showed that the independent predictors of postoperative complications were obesity, COPD, and open appendectomy.

Margenthaler et al.[8] identified the predictors of postoperative morbidity and mortality in patients undergoing surgical intervention for appendicitis based on the ACS NSQIP database. Predictors of morbidity included advanced age, ASA class ≥3, "partially dependent" status, history of COPD, weight loss of >10% within 6 months, and preoperative blood urea nitrogen, bilirubin, albumin, and leukocyte count as well as an infected or contaminated wound and longer operative duration. A retrospective study by Renteria et al.[9] comparing appendectomies in 195 young patients and 62 elderly patients investigated the predictors of complications. Surgery with an open approach, as shown in this study, and a positive cardiac history were reported to independently predict complications, regardless of age. These results are consistent with the findings by Masoomi et al.[6], which compared laparoscopic and open appendectomy in the elderly, and found that compared with the laparoscopic approach, open appendectomy was associated with an increased likelihood of postoperative complications.

In the present study, patients with postoperative complications had a higher prevalence of obesity, hypertension, and

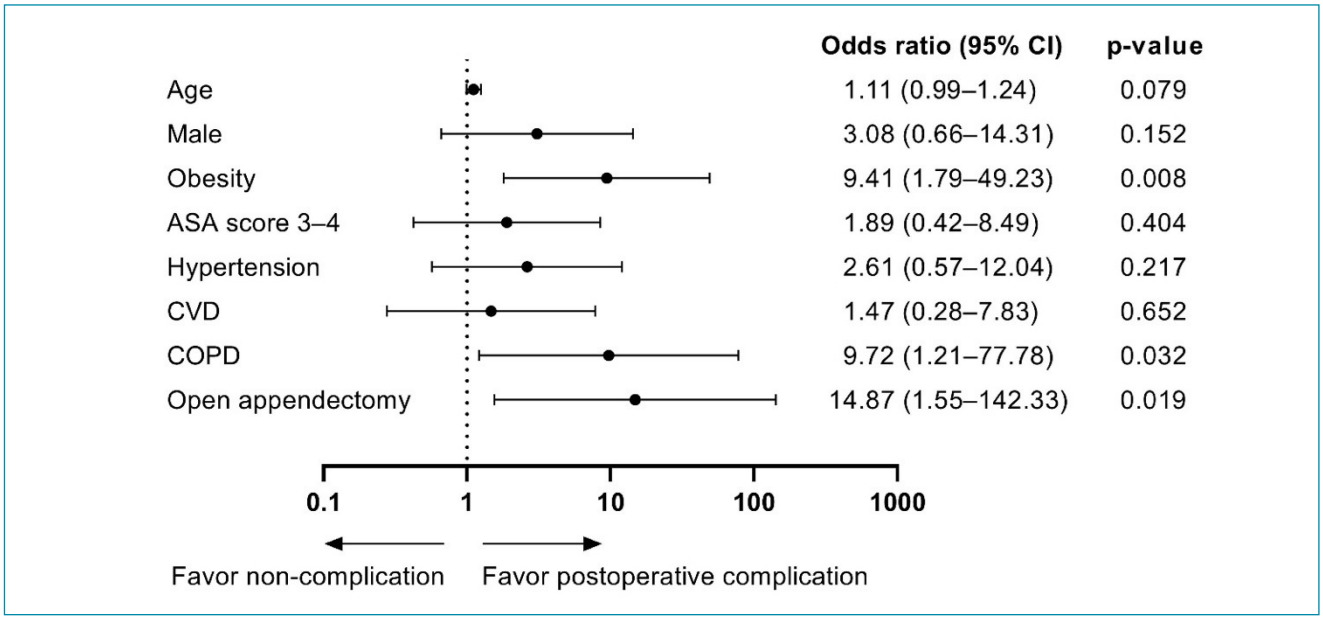


Figure 1. ASA: American Society of Anesthesiologists; COPD: chronic obstructive pulmonary disease; CVD: cardiovascular disease. Forest plot of significant factors in the multivariate analysis for 30-day postoperative complications in elderly patients undergoing appendectomy.

CVD in univariate analysis. However, these significant differences disappeared in the multivariate analysis when covariates were taken into consideration. Contrary to expectations, logistic regression analysis demonstrated that, compared with uncomplicated appendicitis, a complicated appendicitis did not predict increased 30-day complication rates. Other studies have identified several potential factors that may contribute to postoperative complications. Cohen-Arazi et al.[2] showed that a history of cardiac disease was the only predictor of perioperative morbidity. In another nationwide prospective cohort study[12] of 135 patients aged ≥65 years, only renal insufficiency was found to be a significant predictor, regardless of age, complicated or uncomplicated appendicitis, and other comorbid conditions.

In this study, only 27.5% of patients were treated with a laparoscopic approach. This rate appears to be low compared to the literature, which shows the increased use of laparoscopic surgery for the management of abdominal emergencies over the past two decades, with special regard to acute appendicitis.[3,7,10,15] Relevant studies have confirmed that laparoscopic appendectomy is beneficial in terms of shorter recovery time and length of hospital stay and it reduced pain and incidence of wound complications compared to the open approach.[6,10,16] In the present study, open appendectomy was identified as one of the independent predictors of 30-day postoperative complications. This may be interpreted as open appendectomy was more preferred in cases such as complicated appendicitis, peritonitis, or abdominal adhesion. However, this study did not show a significant association between the preferred surgical technique and whether the patient had complicated or uncomplicated appendicitis. Therefore, this reflects the invasive and technically demanding nature of the open approach rather than a selection bias.

This study has several potential limitations. It was limited by the imperfections inherent in any retrospective analysis. The number of patients in this study was also relatively low, although the sample size was similar to other studies evaluating elderly patients with appendicitis, only 5–10% of cases occur in the elderly population. In addition, the present study included a cohort of patients from a single institution, which may lead to selection bias and limits the generalizability to other clinical settings. Finally, the inability to identify patients who were readmitted to a different hospital may have caused an underestimation of the readmission and complication rates. Despite these limitations, this study provides evidence for the understanding and identification of risk factors that predict postoperative outcomes for elderly patients.

## CONCLUSIONS

It is critical to identify modifiable factors that can be addressed preoperatively to reduce the possibility of adverse outcomes and to ensure optimal health outcomes in this frail patient population, potentially leading to decreased resource utilization and economic

cost for the health care system. The study results showed that obesity, COPD, and open appendectomy were independent risk factors for 30-day postoperative complications after the surgical treatment of appendicitis. Hence, laparoscopic appendectomy should be considered an effective and safe procedure for elderly patients with acute appendicitis. Furthermore, elderly patients with comorbidities should be given prompt care to avoid complications.

## AUTHORS' CONTRIBUTIONS

**EL:** Conceptualization, Data curation, Project administration, Writing – original draft, Writing – review & editing. **AD:** Conceptualization, Data curation, Project administration, Writing – original draft, Writing – review & editing. **SUC:** Conceptualization, Data curation, Project administration, Writing – original draft, Writing – review & editing

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# Evaluation of the olfactory bulb volume and morphology in patients with coronavirus disease 2019: can differences create predisposition to anosmia?

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## SUMMARY

**OBJECTIVE:** This study aimed to investigate whether the volume and morphology of the olfactory bulb are effective in the occurrence of anosmia in patients after COVID-19 infection.

**METHODS:** The olfactory bulbus volume was calculated by examining the brain magnetic resonance imaging of cases with positive (+) COVID-19 polymerase chain reaction test with and without anosmia. Evaluated magnetic resonance imaging images were the scans of patients before they were infected with COVID-19. The olfactory bulbus and olfactory nerve morphology of these patients were examined. The brain magnetic resonance imaging of 59 patients with anosmia and 64 controls without anosmia was evaluated. The olfactory bulb volumes of both groups were calculated. The olfactory bulb morphology and olfactory nerve types were examined and compared between the two groups.

**RESULTS:** The left and right olfactory bulb volumes were calculated for the anosmia group and control group as 47.8±15/49.3±14.3 and 50.5±9.9/50.9±9.6, respectively. There was no statistically significant difference between the two groups. When the olfactory bulb morphology was compared between the two groups, it was observed that types D and R were dominant in the anosmia group ($p<0.05$). Concerning olfactory nerve morphology, type N was significantly more common in the control group ($p<0.05$).

**CONCLUSIONS:** According to our results, the olfactory bulb volume does not affect the development of anosmia after COVID-19. However, it is striking that the bulb morphology significantly differs between the patients with and without anosmia. It is clear that the evaluation of COVID-19-associated smell disorders requires studies with a larger number of patients and a clinicoradiological approach.

KEYWORDS: COVID-19. Anosmia. Olfactory bulb. Olfactory nerve. Olfactory mucosa. Epithelium.

## INTRODUCTION

Although coronavirus mainly targets the respiratory system, it can also spread from the respiratory tract to the central nervous system due to its neuroinvasive ability[1]. Therefore, patients with coronavirus disease 2019 (COVID-19) may present with a variety of neurological symptoms such as ischemic infarction, meningitis, encephalitis, bleeding, acute hemorrhagic necrotizing encephalopathy, cerebral venous thrombosis, and diffuse leukoencephalopathy with microhemorrhage[1-4]. Olfactory dysfunction starts suddenly in most cases and is usually temporary, with the recovery time ranging from 1–3 weeks[5].

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Chemosensory symptoms may manifest as viral prodromes or codevelop with other disease symptoms. However, a significant relationship between COVID-19 and sinonasal symptoms has not yet been detected, suggesting that the pathogenesis of anosmia may differ from obstructive olfactory dysfunction that is seen in other viral upper respiratory tract infections[6,7].

The olfactory bulb (OB) is located above the cribriform plate just below the olfactory sulcus (OS) in the anterior cranial fossa and is easily recognizable on conventional magnetic resonance imaging (MRI). The olfactory neural network is connected to the piriform cortex and amygdala through first-order projections and to the orbitofrontal cortex, thalamus, and insula through secondary projections[8,9].

There is limited literature on OB imaging in COVID-19 olfactory dysfunction, with the availability of only a few case reports[10,11]. Abnormal findings reported include microhemorrhage in OB, signal abnormality, increased enhancement, and enlarged or reduced atrophied OB[9,12].

To the best of our knowledge, there is no study in the English language literature evaluating the effect of OB and OS morphology on the frequency of anosmia in patients with COVID-19. This study aimed to determine whether the OB volume and OB and OS morphologies were associated with the frequency of anosmia in COVID-19-positive patients.

# METHODS

## Patient selection

This study was approved by the Ethical Committee and conducted in full accordance with the guidelines of the Declaration of Helsinki. This study was retrospective, and 7,538 patients who were admitted to our hospital's COVID Outpatient Clinic between April 2020 and December 2020 and who were positive for COVID-19 according to the polymerase chain reaction test (PCR) were screened. A total of 123 patients who had brain MRI before the development of anosmia were included in this study. Patients with brain MRI examination older than one year were excluded from this study, as the reliability of clinical information may be impaired.

Out of these, 59 patients, whose olfactory dysfunction still persisted despite the improvement of other COVID-19-related symptoms, were selected for the anosmia group.

There were no other reasons that could cause smell disorders in the patients in the anosmia group. Patients with a history of neurodegenerative disease were excluded from this study considering that their anosmia might not have been associated with COVID-19[13,14]. In addition, patients with a Kennedy staging of other than 0 according to the paranasal sinus MRI examination and those with a history of chronic rhinosinusitis were excluded since these conditions could cause anosmia[15,16]. Finally, pediatric patients, pregnant women, patients with a previous history of loss or changes in smell and taste, and those with allergic rhinitis, a history of head and neck trauma or migraine, and pathological signal changes in the amygdala and orbitofrontal cortex on brain MRI were also excluded from this study. As the control group, 64 people who did not complain of loss of smell during or after COVID-19 and who had brain MRI in the last year due to nonspecific headache and vertigo were selected.

## MRI acquisition

MRI examination was performed on a 1.5 T unit (Philips Ingenia, Best, Eindhoven, Netherlands, 2017). The technical parameters were as follows: axial T1-weighted [repetition time (TR): 550–750 ms; echo time (TE): 20–25 ms; scan thickness: 5 mm; slice gap: 1 mm; and matrix: 256×256], axial T2-weighted (TR: 4,000–5,000 ms; TE: 90–120 ms; scan thickness: 5 mm; slice gap: 1 mm; and matrix: 256×256), sagittal T2-weighted fluid-attenuated inversion recovery (FLAIR) (TR: , 7,200 ms; TE: 120 ms; FA: 90°; TI: 1,333–2,041 ms; and matrix, 256×256), and coronal T2 (TR: 6,550 ms; TE: 99 ms, flip angle: 150°; slice thickness: 5 mm; and matrix: 256×256). All images were evaluated using the Philips IntelliSpace workstation.

## MRI evaluation

The OS depth was measured on coronal T2 images to the deepest point of the OS by drawing a tangent line to the lower boundaries of the gyrus rectus and medial orbital gyrus[17]. The OB volume and morphology were evaluated in coronal T2-weighted sections. Since most pathologies can affect the ipsilateral gyrus rectus and OB simultaneously, the reference point was taken as the corticomedullary signal intensity of the contralateral gyrus rectus. The oval or inverted J-shaped OBs were considered normal (type N) (Figure 1A). Shrunken or flattened OBs without deformity were accepted as type R[18,19] (Figures 1B and 1D). The presence of asymmetric contour lobulation or hyperintense focus of >1 on T2 images was accepted as type D[17-19] (Figure 1C). The olfactory nerve was evaluated using the sagittal FLAIR sequence. Thin and straight stretched fibers that were evenly aligned were considered normal (type N)[18-20] (Figures 2A and 2B). Non-uniform olfactory nerves with an irregular inferior projection in the lower contour of OB were considered as type C while those with markedly thinned calibration were classified as thinning and scarcity (TS)[19-21] (Figures 2C and 2D). The evaluations were performed based on the consensus of three radiologists, blinded to the clinical information of the patients. In case of disagreement, an experienced radiologist's opinion was sought.

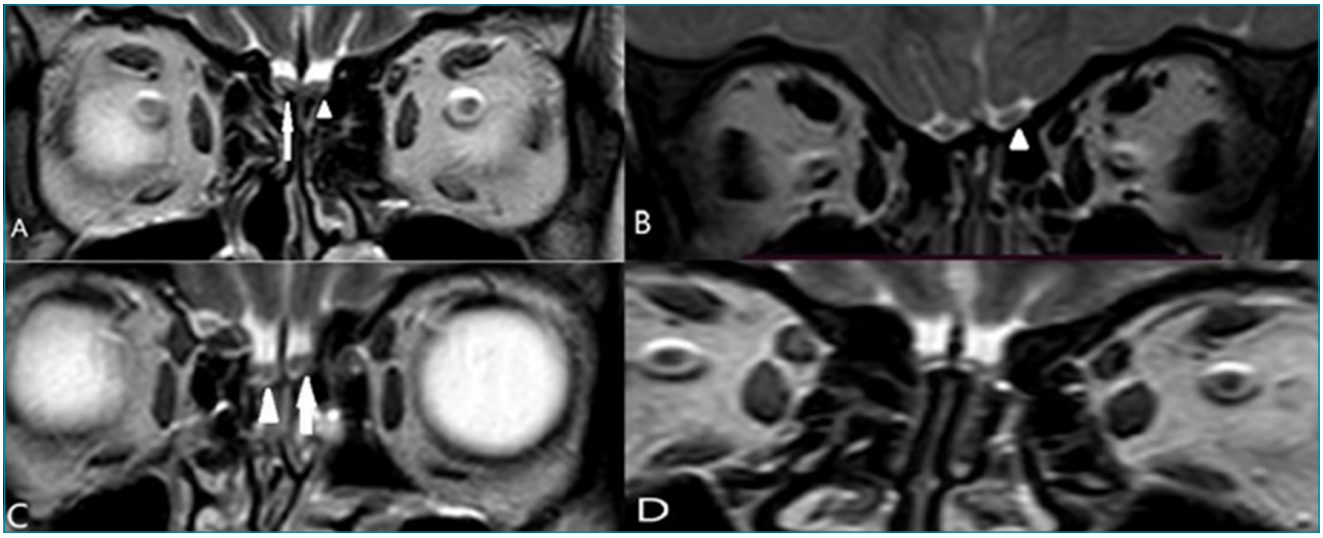


Figure 1. (A and B): Coronal T2-weighted examination shows that the right olfactory bulbus is seen as inverted j and was considered to be normal type (long arrow), and the left olfactory bulbus appears flattened and shrunken and was considered type R (arrowhead). (C): Coronal T2-weighted examination shows that the right olfactory bulbus shows more than 1 hyperintense focus and asymmetric contour lobulation (arrowhead), and the left olfactory bulbus shows contour lobulation (arrow) both were considered type D. (D): Coronal T2-weighted examination shows that both olfactory bulbus were considered flattened and type R.

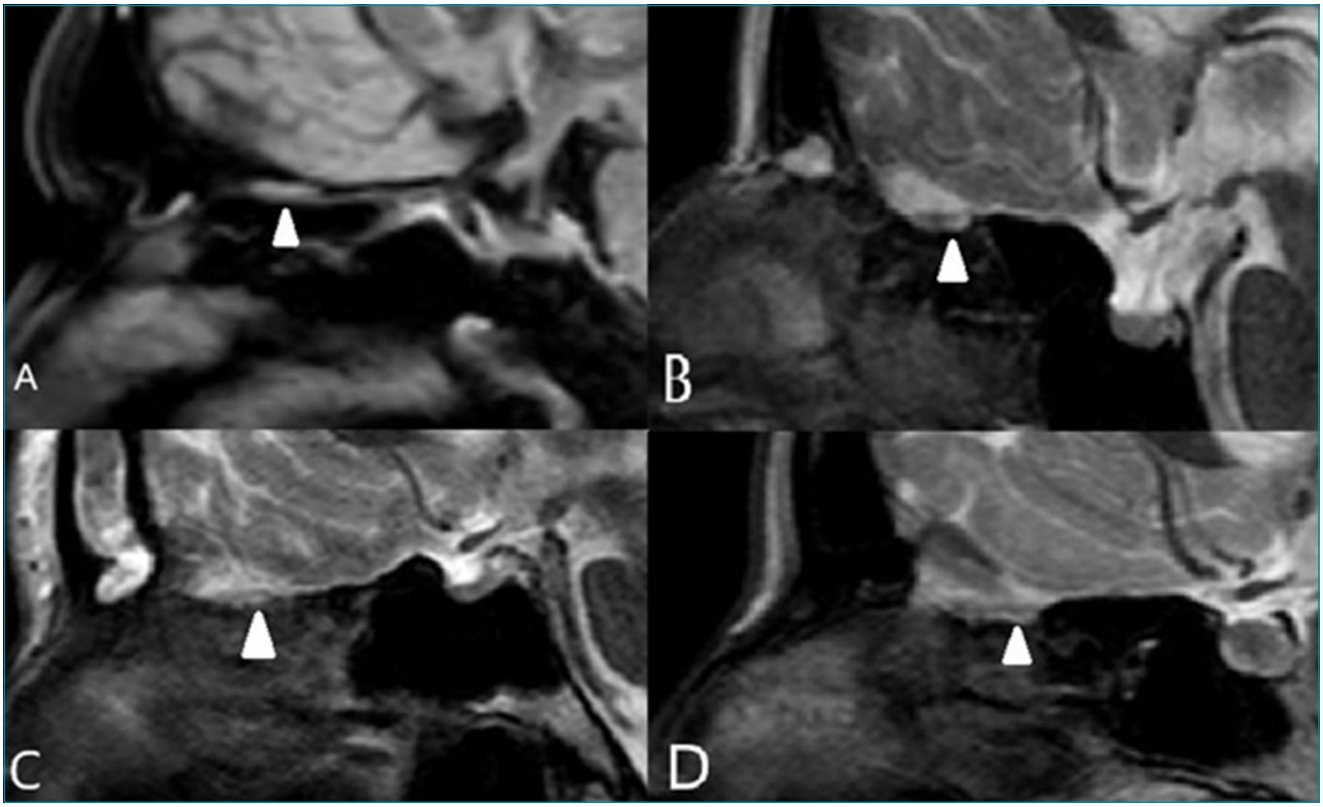


Figure 2. (A): Sagittal fluid-attenuated inversion recovery and (B): saggital T2-weighted examination show that smooth and thick olfactory nerve was accepted as type N. (C): Saggital T2-weighted examination shows that the significantly thinned and deformed olfactory nerve was accepted as type thinning and scarcity (olfactory nerves marked with arrowhead). (D): Sagittal fluid-attenuated inversion recovery weighted examination shows that nonuniform olfactory nerve with thinned and deformed appearance was accepted as type thinning and scarcity (olfactory nerves marked with arrowhead).

### Statistical analysis

The Statistical Package for the Social Sciences (SPSS) version 23.0 software package was used for the statistical analysis of the data. Categorical measurements were summarized as numbers and percentages and continuous measurements as mean and standard deviation values (median and minimum–maximum where necessary). The Shapiro-Wilk test was used to determine whether the parameters in this study showed a normal distribution. In the comparison of continuous measurements between the groups, the normality of distribution was checked, the Mann–Whitney U test was used in binary variables for parameters that did not show a normal distribution, and the independent Student's *t*-test was used for paired group analyses for normally distributed data. The statistical significance level was 0.05 in all tests.

## RESULTS

In this study, a total of 123 brain MRIs taken before the COVID-19 PCR (+) positivity, 59 belonging to the anosmia group and 64 to the control group, were retrospectively analyzed. There were 33 men and 26 women in the anosmia group and 33 men and 31 women in the control group. The mean age was 54.5 (21–71) years for the anosmia group and 55 (19–80) years for the control group. There was no statistically significant difference in age and gender between the two groups (p=0.29 and 0.627, respectively) (Table 1).

No statistically significant difference was found between the anosmia and control groups in relation to the left and right OB volumes (p=0.236 and 0.467, respectively). Similarly, there was no statistically significant difference in the left and right OS depths between the two groups (p=0.92 and 0.374, respectively) (Table 1; Figure 3).

Table 1. Statistical analysis of the demographic data and olfactory bulb and olfactory bulb parameters of the study groups.

| | Anosmia (n=59) | Control (n=64) | p-value |
|---|---|---|---|
| Gender | | | |
| Male | 33 (55.9) | 33 (51.6) | 0.627 |
| Female | 26 (44.1) | 31 (48.4) | |
| Age | 54.5 (21–71) | 55 (19–80) | 0.293 |
| olfactory bulb volume, left | 47.8±15.0 | 50.5±9.9 | 0.236 |
| olfactory bulb volume, right | 49.3±14.3 | 50.9±9.6 | 0.467 |
| olfactory sulcus depth, left | 6.75 (2.8–9.8) | 6.75 (3.1–10.5) | 0.922 |
| olfactory sulcus depth, right | 6.65 (3–10) | 6.72 (3–11) | 0.374 |

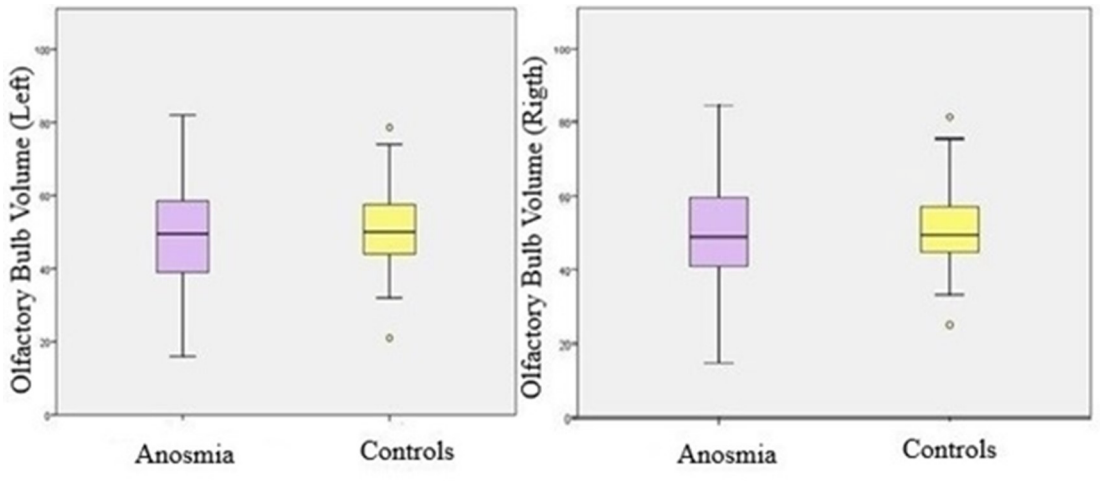


Figure 3. Box and whisker plots showing the median and interquartile range values for each group. No statistically significant difference was found between anosmia and control groups in terms of olfactory bulbus volumes.

When the OB types were compared between the anosmia and control groups, types D and R were more common in the anosmia group with a statistically significant difference. Type J was found at a higher rate in the control group compared with the anosmia group, and this was at a statistically significant level (Table 2).

When the distribution of olfactory nerve morphology was examined in both groups, type N was more common in the control group at a statistically significant rate. In contrast, the rates of patients with types C and TS did not differ between the anosmia and control groups (Table 2).

## DISCUSSION

The pathogenesis of olfactory dysfunction in COVID-19 disease is not yet fully understood; however, studies have shown no significant association between sinonasal symptoms and COVID-19[22]. According to a hypothesis, in COVID-19, anosmia is caused by the virus entering the central nervous system through olfactory sensory neurons in the olfactory mucosa[23]. It has also been previously shown that COVID-19 can migrate from the nose to OB in an experimental mouse model[24]. The possible mechanisms that are most frequently considered in the pathogenesis of COVID-19 anosmia are olfactory cleft inflammation/occlusion and/or OB damage[23,25].

Table 2. Statistical distribution and analysis of olfactory bulb and olfactory nerve types in the study groups.

| | | Anosmia (n=59) | Control (n=64) | p-value |
|---|---|---|---|---|
| olfactory bulb types, left | | | | |
| | D | 16 (27.1) | 8 (12.5) | **0.041** |
| | J | 22 (37.3) | 43 (67.2) | **0.003** |
| | R | 21 (35.6) | 13 (20.3) | **0.045** |
| olfactory bulb types, right | | | | |
| | D | 15 (25.4) | 7 (10.9) | **0.036** |
| | J | 18 (30.5) | 40 (62.5) | **0.001** |
| | R | 26 (44.1) | 17 (26.6) | **0.042** |
| Olfactory nerve types, left | | | | |
| | C | 26 (37.3) | 19 (29.7) | 0.098 |
| | TS | 22 (18.6) | 15 (23.4) | 0.094 |
| | N | 11 (81.4) | 30 (46.9) | **0.001** |
| Olfactory nerve types, right | | | | |
| | C | 23 (35.6) | 18 (28.1) | 0.202 |
| | TS | 21 (25.4) | 15 (23.4) | 0.139 |
| | N | 15 (74.6) | 31 (48.4) | **0.008** |

Bold values are statistically relevant.

Angiotensin-converting enzyme 2 (ACE2) receptors, target molecules for COVID-19, are expressed by non-neuronal support cells of the olfactory epithelium but not directly by olfactory neurons. Anosmia may result from injury to the supporting cells of the epithelium. This is supported by postviral anosmia studies in which prolonged or persistent anosmia reflects the olfactory epithelial regeneration interval[24].

Evaluating imaging findings in patients with anosmia can be very complex. In such evaluations, OB volume and OS depth were considered the most effective and were the most frequently measured parameters[21,23]. Although exact values have not been determined in studies on OB volume, the common consensus is that the normal OB volume is >45 mL, and the normal OS depth is >7 mm[13,20,22].

The literature shows that OB volume loss is mainly detected in the idiopathic or post-viral anosmia groups[11,12,22]. In this study, no significant difference was found in the pre-disease OB volumes of the anosmia and control groups. Despite the presence of contradictions in the findings and the lack of a complete consensus in the literature, our results indicate that OB volume may not really be the main cause of anosmia after COVID-19. Similar studies, prospective studies if possible, with larger series are needed to clarify this issue.

The data in this study showed that the risk of anosmia was increased among the patients with type D and R OBs. The OB volume did not statistically significantly differ between the study groups while the difference in the OB type was statistically significant, suggesting that nerve morphology rather than volume might be effective in anosmia. It is clear that there is still a need to ascertain why the types of OB morphology result in a difference in olfactory function.

In light of these data, our hypothesis is the possibility of differences in primary neurons in the olfactory mucosa accompanying morphological types D and R that can be defined by MRI.

When the literature is examined, there is no hypothesis as to why the morphological type is effective in the loss of smell after COVID-19. However, we have a few hypotheses to explain this situation. The strongest of these hypotheses is that nerves may have varied in their morphology as well as the surface areas where they terminate in the cribriform plates. Thus, the relationship between the variability of angiotensin-converting enzyme 2 receptor (ACE2) and transmembrane protease serine two receptor density and morphology can be explained[24,25]. Postmortem autopsy studies are needed to evaluate the validity of this hypothesis, as primary sensory neurons in the olfactory mucosa cannot be visualized by MRI. Another hypothesis is that morphology is important only in the patient group we

evaluated. Multicenter, multi-participant studies are needed to confirm whether this is coincidental or whether morphology actually has meaning.

When the olfactory nerve types were compared between the anosmia and control groups, types C and TS, which are less common in the general population, were not statistically associated with olfactory dysfunction. However, type N, which is the most common type and indicates normal morphology, was seen at a significantly higher rate in the control group without anosmia. We consider that similar studies to be conducted with a higher number of patients may present comprehensive data on this subject.

There were some limitations to this study. We consider the major limitation to be the relatively small sample size due to the strict criteria used in patient selection. Other limitations include the retrospective nature of the study and the absence of an interobserver evaluation.

For the diagnosis of anosmia, the complaints of the patients were taken as a basis, and no objective test was used. It would be ideal to use an orbital MRI to evaluate the OB; however, it would be very difficult to achieve this in pandemic conditions. Therefore, brain MRI was used, and this can be considered as another limitation.

## CONCLUSIONS

It is commonly accepted that COVID-19 affects OB, but it has not yet been elucidated how this effect on OB causes olfactory dysfunction. In this study, we observed that the OB volume before COVID-19 infection had no significant effect on the etiology of anosmia development during or after the disease. However, it is considered that the morphology of the OB type and primary neurons in the accompanying olfactory mucosal epithelium may play a key role in olfactory dysfunction, and there is a need for further studies to shed light on this subject.

## AUTHORS' CONTRIBUTIONS

**HA**: Conceptualization, Data curation, Formal Analysis, Writing – original draft, Writing – review & editing. **OD**: Conceptualization, Data curation, Formal Analysis, Writing – review & editing. **ÖK:** Conceptualization, Data curation, Formal Analysis, Writing – review & editing. **CY:** Conceptualization, Data curation, Formal Analysis, Writing – review & editing. **CB**: Conceptualization, Data curation, Formal Analysis, Writing – review & editing. **AK:** Conceptualization, Data curation, Formal Analysis, Writing – review & editing. **BG:** Conceptualization, Data curation, Formal Analysis, Writing – review & editing. **CB:** Writing – review & editing.

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# C-reactive protein and neutrophil–lymphocyte ratio as predictors of mortality in coronavirus disease 2019

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## SUMMARY

**OBJECTIVE:** This study investigates whether C-reactive protein, platelet–lymphocyte ratio, and neutrophil–lymphocyte ratio could be useful to predict mortality in COVID-19.

**METHODS:** Data of 635 patients with COVID-19 followed up in Sinop Ataturk State Hospital from February to May 2020 were evaluated retrospectively. Diagnosis of COVID-19 was made according to the interim guidance of the World Health Organization. Patients were grouped into two groups based on mortality as survived and non-survived patients. Age, gender, neutrophil–lymphocyte ratio, platelet–lymphocyte ratio, and C-reactive protein of the groups were investigated and compared.

**RESULTS:** The mean age of the participants was 55.8±22.3 years. Among the patients, 584 survived and 51 patients died. Age was significantly different between the groups, 54.2±22.3 in the survived group and 75.6±11.1 in the dead group (p=0.000). In addition, neutrophil, C-reactive protein, and neutrophil–lymphocyte ratio values were significantly higher in the dead group (p=0.000). platelet–lymphocyte ratio was slightly higher in the dead group, but this difference was not significant (p=0.42). The area under the curve values for age, lymphocyte, platelet, C-reactive protein, and neutrophil–lymphocyte ratio are 0.797, 0.424, 0.485, 0.778, and 0.729, respectively.

**CONCLUSIONS:** Our results showed that neutrophil–lymphocyte ratio and C-reactive protein are significantly higher in patients leading to death and could be effective biomarkers in predicting COVID-19 fatality. Furthermore, C-reactive protein could be used as an independent biomarker to predict death in patients with COVID-19, regardless of gender and age (p=0.000).

KEYWORDS: COVID-19. C-Reactive Protein. Neutrophil. Lymphocyte. Platelet. Mortality.

## INTRODUCTION

The first case of the spread of abnormal pneumonia was observed on December 29, 2010, in Wuhan, China, and the first case of which was discovered on December 12 in the same year[1]. Later, an abnormal outbreak was reported to the World Health Organization (WHO) on December 31. After various speculations about the origin of the disease, China CDC has introduced a new coronavirus called 2019-novel coronavirus disease (nCoV-2019) or COVID-19[2]. The first nCoV-2019 genomic sequence went online one day after Zhang et al. approved it at Fudan University in Shanghai[3]. Isolation and successful genomic sequencing of COVID-19 have helped understand the virus's origin and its infectious properties[4]. The new coronavirus outbreak has been declared a public health emergency worldwide, posing a threat to China and all countries[5]. However, many ambiguities remain, and scientists are conducting extensive research on this new virus.

If the disease progresses, it will cause the immune system to overreact[4,6]. The chemical signals of cytokines cause

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inflammation that must be regulated[2]. Inflammation of the lungs causes pneumonia, leading to multiple organ failure and subsequent mortality[7]. If the immune system fails to resist the virus, it spreads to every organ of the body, causing further damage[3]. Inflammatory processes usually cause changes in the body's biomarkers that can be measured to determine the state of inflammation and subsequent prognosis[8]. Some of these biomarkers are the neutrophil–lymphocyte ratio (NLR), platelet–lymphocyte ratio (PLR), and C-reactive protein (CRP), which are used as biomarkers predicting pneumonia. This study aimed to investigate the relationship of NLR, PLR, and CRP with COVID-19 mortality.

## METHODS

This is a retrospective study of data from 635 patients with COVID-19 referred to Sinop State Hospital from March to November 2020. The diagnosis of COVID-19 was made according to the WHO interim guidance. Only patients whose COVID-19 were confirmed by the laboratory participated in this study. Fifty-one participants died, and 584 patients survived. Patients were grouped into two groups based on fatality. Due to anonymous, retrospective, and observational nature of this study, patients' informed consent was waived.

Exposure history records, clinical signs, epidemiological characteristics, and laboratory data of patients were obtained from their electronic records and telephonic confirmation. Admission white blood cell (WBC), lymphocyte (LYM), neutrophil (NEU), NLR, platelet, PLR, and CRP were important variables selected from patients' records. Other laboratory data obtained from the records included complete blood count and blood chemistry. Based on the patient's death or survival, this study group was divided into two groups. The exitus group included 51 patients, and the survivor group included 584 patients.

$\chi^2$ test and Fisher's exact test were used to compare categorical variables, while Wilcoxon rank-sum test was used to compare continuous variables. Received operational curve (ROC) analysis was used to obtain the optimal cutoff values of PLR, CRP, and NLR variables to determine the mortality. 95% confidence interval (CI) and hazard risk (HR) were used to assess the relevant risks. Binary logistic regression analysis was used to determine the effect of age, gender, and some other factors on mortality. $p<0.05$ was considered as a statistically significant value. All statistical calculations were performed using SATA 14 software.

## RESULTS

Demographic information and clinical characteristics of patients are given in Table 1. The mean age of the participants was 55.8±22.3 years. This value was significantly different between the groups (p=0.000), 54.2±22.3 in the survived group and 75.6±11.1 in the exitus group. There was a significant difference between the survivor and the exitus groups with respect to gender, and men had a higher death rate than women (p=0.008). The WBC was 9.4±31.6 in the survivor group and 12.6±7.04 in the exitus group, which was significantly higher than the exitus group (p=0.000). NEU, CRP, and NLR values were also significantly higher in the exitus group (p=0.000). PLR was a little higher in the exitus group, but this difference was not significant (p=0.42).

To determine the relationship between these biomarkers and COVID-19 fatality, the optimal cutoff values were calculated by ROC analysis, and the results are shown in Table 2. As shown in Table 2, the area under the curve (AUC) values for age, LYM, platelet, CRP, and NLR are 0.797, 0.424, 0.485, 0.778, and 0.729, respectively. LYM and platelet levels cannot be used as diagnostic biomarkers for patients' risk of

Table 1. Characteristics of the study population.

| | Total | Survived | Dead | p-value |
|---|---|---|---|---|
| Age (M±SD) | 55.8±22.3 | 54.2±22.3 | 75.6±11.1 | 0.000 |
| Sex (M/F) | 322/313 | 287/297 | 35/16 | 0.008 |
| WBC (M±SD) | 9.6±29.8 | 9.4±31.6 | 12.6±7.04 | 0.000 |
| LYM | 2.01±7.5 | 2.04±8.02 | 1.7±2.5 | 0.1 |
| NEU | 7.09±15.7 | 6.9±16.5 | 10.4±5.9 | 0.000 |
| Platelet | 224.4±85.8 | 224.4±82.3 | 230.1±106.4 | 0.9 |
| CRP | 48.2±63.9 | 43.08±58.6 | 112.4±91.2 | 0.000 |
| NLR | 6.1±12.01 | 5.9±12.3 | 10.8±9.6 | 0.000 |
| PLR | 182.3±150.8 | 180.02±147.5 | 229.1±198.7 | 0.42 |

M: mean; SD: standard deviation; M: male; F: female; WBC: white blood cell; LYM: lymphocyte; NEU: neutrophil; CRP: C-reactive protein; NLR: neutrophil–lymphocyte ratio; PLR: platelet–lymphocyte ratio.

death because their AUC<0.50. However, Table 2 shows that CRP and NLR could be used as diagnostic biomarkers for COVID-19 fatality.

The Kaplan–Meier curve and the univariate Cox regression model were used to examine the factors that could lead to the death from COVID-19. The variables of NLR and PLR were included in univariate analyses to determine their effect on the death of patients with COVID-19. The analysis result shows that NLR can be considered an independent factor associated with the death of patients with COVID-19. However, PLR did not show any correlation with COVID-19 fatality.

The crude odds ratio (OR) was calculated through logistic regression analysis to evaluate the predictability of death due to COVID-19 by the investigated parameters (Table 3). Due to the age and gender effect on parameters, their effect was adjusted and presented in a separate column. The OR p-values of CRP, NLR, and PLR were 0.000, 0.004, and 0.02, respectively. As shown in Table 3, CRP, NLR, and PLR can effectively predict mortality by COVID-19 by considering age and gender. However, only the adjusted odds ratio (ORa) CRP was p>0.05, which means that only CRP could be used as an independent biomarker to predict death in COVID-19 patients, regardless of age and gender (p=0.000).

## DISCUSSION

COVID-19, also commonly known as coronavirus, is an infectious disease caused by coronavirus (2019-nCoV) acute respiratory disease infections[9,10]. Our knowledge of this disease is incomplete and is developing. Also, coronaviruses are often known to combine mutations and openings, posing an ongoing challenge to our understanding and clinical management.

Apart from clinical symptoms, immunological features in patients can be warning signs of disease deterioration. This study showed that increased NLR in patients could sign the progression of pneumonia and an increased risk of death in patients with COVID-19. This finding was consistent with previous studies[11-17]. The relationship between NLR and infectious diseases is well-known. An explanation for this relationship may be that the NEU is a part of leukocytes that arises from the venous system and is transmitted to the immune system[11]. NEU generates large amounts of reactive oxygen species and could save the cell from the virus by inducing DNA damage[12]. Our results showed that increased NLR is a sign of COVID-19 progress and can lead to more severe disease and eventually death. In this study, a threshold of 3.3 was considered for NLR *via* the ROC, which showed that it could predict the severity of the disease well, and these results are consistent with the findings of other studies[13-15].

Table 2. Area under the curve values of age, lymphocyte, platelet, C-reactive protein, and neutrophil–lymphocyte ratio.

| Test result variable(s) | Area | Standard error[a] | Asymptotic Significance[b] | Asymptotic 95%CI | |
|---|---|---|---|---|---|
| | | | | Lower bound | Upper bound |
| Age | 0.797 | 0.030 | 0.000 | 0.739 | 0.855 |
| LYM | 0.424 | 0.054 | 0.094 | 0.318 | 0.530 |
| Platelet | 0.485 | 0.054 | 0.743 | 0.380 | 0.590 |
| CRP | 0.778 | 0.035 | 0.000 | 0.708 | 0.847 |
| NLR | 0.729 | 0.040 | 0.000 | 0.651 | 0.808 |

[a]Under the nonparametric assumption. [b]Null hypothesis: true area=0.5. AUC: area under the curve; LYM: lymphocyte; CRP: C-reactive protein; NLR: neutrophil–lymphocyte ratio.

Table 3. The crude odds ratio and adjusted odds ratio for variables.

| Indicators | OR | p-value | ORa* | p-value |
|---|---|---|---|---|
| WBC | 1 (0.99–1) | 0.1 | 1 (0.97–1.02) | 0.9 |
| LYM | 0.99 (0.91–1.08) | 0.9 | 0.99 (0.95–1.04) | 0.8 |
| NEU | 1 (0.99–1.01) | 0.08 | 0.99 (0.94–1.04) | 0.9 |
| Platelet | 1 (0.99–1) | 0.6 | 1.001 (0.99–1.004) | 0.2 |
| CRP | 1.008 (1–1.01) | 0.000 | 1.007 (1.004–1.01) | 0.000 |
| NLR | 1.01 (1–1.02) | 0.004 | 1.01 (0.93–1.1) | 0.8 |
| PLR | 1 (1–1.002) | 0.02 | 0.99 (0.99–1.003) | 0.7 |

*Adjustment for age and gender. WBC: white blood cell; LYM: lymphocyte; NEU: neutrophil; CRP: C-reactive protein; NLR: neutrophil–lymphocyte ratio; PLR: platelet–lymphocyte ratio.

Another notable biomarker in our results was CRP, which is positively correlated with the level of inflammation in the body[18]. Studies have shown that factors such as gender, age, or physical condition of the patient do not impact the CRP concentration level[18-20], which is consistent with our findings. This study found that CRP could be used as an independent biomarker for COVID-19 fatality, regardless of age and gender. Previous studies have shown that CRP levels can be used for the early detection of patients with pneumonia, which had higher levels of CRP than others[18,21]. In line with these findings, in this study, CRP levels were significantly correlated with disease severity and patient death. This means that CRP levels can be considered a warning factor for the progression and mortality by COVID-19.

One of the limitations of this study is that the study data are taken from a single clinical research center, which may reduce the accuracy of conclusions due to demographic and local conditions. Future studies are needed to demonstrate the results of several clinical research centers with different demographic and influential clinical data.

## CONCLUSIONS

Our results showed that NLR and CRP are significantly higher in patients who died from COVID-19, and they could be effective biomarkers in predicting COVID-19 mortality. Our results also showed that CRP could be used as an age- and gender-independent biomarker to predict disease progression and mortality.

## AUTHORS' CONTRIBUTIONS

**HE:** Conceptualization, Investigation, Methodology, Project administration, Software, Supervision, Visualization, Writing – original draft. **ZE:** Conceptualization, Methodology, Project administration, Software, Visualization. **MD:** Data curation, Funding acquisition, Resources, Validation. **MU:** Data curation, Funding acquisition, Resources, Validation. **HTG:** Formal Analysis, Investigation, Supervision, Writing – review & editing.

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REVIEW ARTICLE
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# Use of probiotics in pediatric patients with autism spectrum disorder: a systematic review

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## INTRODUCTION

Autism spectrum disorder (ASD) is a neurodevelopmental disorder, characterized by persistent impairment in communication and social interaction, in addition to repetitive and stereotyped behaviors[1]. Approximately 50% of patients with ASD also have gastrointestinal (GI) symptoms, mainly constipation, abdominal pain, diarrhea, flatulence, and vomiting[2].

According to the study by Karimi[3], the etiology of ASD is multifactorial, involving environmental and genetic factors. Recent studies[4-6] indicate dysbiosis of the intestinal microbiota as an important factor in its development and in other neuropsychiatric diseases, such as depression and Parkinson's disease[7].

A review of the literature showed that children with ASD have a greater abundance of *Bacteroides*, *Parabacteroides*, *Clostridium*, *Faecalibacterium*, and *Phascolarctobacterium* colonizing them, differing from the pattern of colonization of neurotypical children who generally have a higher prevalence of *Coprococcus* and *Bifidobacterium*[8].

Defined by the World Health Organization (WHO) as "Live microorganisms that, when administered in adequate quantities, confer a health benefit,"[9] probiotics have gained prominence after studies suggest that they could be a useful therapeutic tool to alter brain function by its activity in restoring the healthy balance of the intestinal microbiota and modulating the levels of neurotransmitters[10-12].

As an example, the hypothalamic-pituitary-adrenal (HPA) stress response, which controls emotion and mood, can be attenuated by certain probiotic microorganisms, thus decreasing the levels of corticosteroids. In the immune system, some probiotics can restrict the production of pro-inflammatory cytokines and can alter metabolites such as tryptophan and short-chain fatty acids, which help to regulate the cellular immune response[13].

This study aimed to provide an updated review in order to clarify the effect of the use of probiotics, when compared to placebo, in the behavioral aspect and in the gastrointestinal tract (GIT) of pediatric patients with ASD.

## METHODS

This systematic review of randomized controlled trials (RCTs) evaluated the effects of probiotics on the behavioral issue and on the GIT of pediatric patients with ASD. The articles were selected according to the recommendations of the Preferred Reporting Items for Systematic Reviews and Meta-analysis (The PRISMA Statement)[14], responsible for coordinating the process of making meta-analyses and systematic reviews.

For the selection, a systematic search of the literature was carried out in the databases Medline (via PubMed), LILACS (via Virtual Health Library), and SciELO. The following search terms were used in the Medline databases: (probiotics) AND (autism OR ASD); LILACS: (("Autistic Spectrum Disorder") AND ("probiotics")); SciELO: (probiotics) AND (autism OR ASD). The surveys were carried out between February and March 2021, without language restrictions.

The inclusion criteria for this review included only the placebo-controlled RCTs, the studies carried out with the pediatric population diagnosed with ASD and intervention with probiotics. The exclusion criteria were observational studies, studies in the non-pediatric population, literature reviews, duplicate studies, interventions without the use of probiotics, and studies with patients with diseases associated with ASD. After a

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systematic review of the literature using the databases mentioned above, the articles that met the inclusion criteria were selected. A total of 215 articles were found as follows: 187 via Medline, 27 via LILACS, and 1 via SciELO. After the methodological screening, two studies were eligible for this systematic review. Figure 1 describes the steps for selecting the articles.

## RESULTS

The two studies selected for this review are RCTs, containing a control group receiving placebo and an intervention group receiving probiotics, in a pediatric population diagnosed with ASD, totalizing 134 patients.

The studies were evaluated individually for methodological quality (risk of bias) following Cochrane's Risk of Bias Tool (RoB-2)[15], which is more detailed in Table 1.

In 2019, to study the impacts of *Lactobacillus plantarum* PS128 on the brain-intestine axis, Liu et al.[16] underwent a four-week double-blind RCT with 71 male patients between 7 and 15 years (36 controls and 35 interventions) diagnosed with ASD. Candidates who had taken antibiotics, yogurts, or probiotic products two weeks prior to registration were excluded. Participants were allowed to continue their regular medications, treatment, and therapies, with the exception of antibiotics, and were asked to refrain from consuming yogurt or probiotic products during the study period. The results were evaluated from the questionnaires, such as Autism Behavior Checklist-Taiwan (ABC-T); Social Responsiveness Scale (SRS); Child Behavior Checklist (CBCL); Multimodal Treatment Study for Attention Deficit Hyperactivity Disorder (ADHD), version of Swanson, Nolan, and Pelham, Version IV, adapted for Brazil (SNAP-IV). After 4 weeks, there was a statistical significance in the reduction of the total scores of SRS (p=0.04) and SNAP-IV (p=0.02) of the group that received treatment with probiotics, a fact not observed in the placebo group. In addition, in the treated group, exploratory analyses revealed improvement in anxiety and rule-breaking behaviors (p=0.02) in CBCL and improvement in relation to body and object use (p=0.04) in the ABC-T. In SNAP-IV, there was an improvement in hyperactivity and impulsivity (p=0.04). No adverse events, GI intolerance, or allergic response were reported by parents or participants.

The study by Santocchi et al.[17] aimed to evaluate the use of the probiotic mixture Vivomixx® in pediatric patients diagnosed with ASD who have GI symptoms and non-GI (NGI) symptoms. The primary outcome was to evaluate the improvement in the level of severity of ASD symptomatology through the Autism Diagnostic Observation Schedule Calibrated Severity Score (ADOS-CSS), and the secondary outcome was to evaluate the improvement of GI symptoms, assessed by the Gastrointestinal Severity Index (GSI). The exclusion criteria were neurological syndromes or focal neurological signs, epilepsy, history of neonatal asphyxia, severe prematurity, and other perinatal lesions; significant sensory deficiency (e.g., blindness and deafness); and diagnosis of nonfunctional GI disorder or celiac disease and special diets already underway. A double-blind and parallel RCT was conducted in 63 children aged 18–72 months, the control group composed of eight GI and 24 NGI children, and the intervention group consisted of nine GI and 22 NGI children. Therapy was applied at a dose

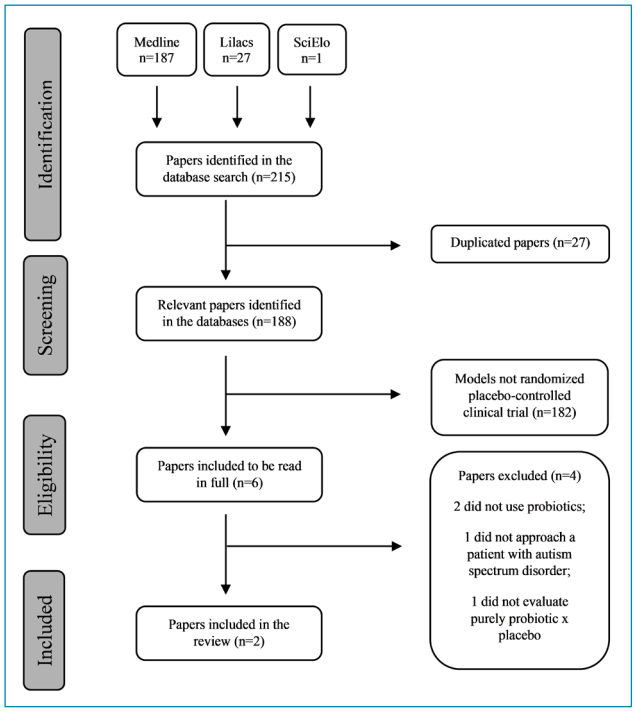


Figure 1. Flowchart of studies included in the analysis.

Table 1. Cochrane's Risk of Bias Tool[15].

| Criteria | Liu et al.[16] | Santocchi et al.[17] |
|---|---|---|
| 1. Clear objective | A | A |
| 2.Suitable sample size | B | B |
| 3.Sample identification and evaluation | A | A |
| 4. Comparability | B | A |
| 5. Blinding of participants | A | A |
| 6. Other bias | C | B |
| 7. Proper statistical analysis | B | B |
| Total | C | C |

A: low risk of bias; B: intermediate risk of bias; C: high risk of bias.

of two sachets/day in the first month and one sachet/day in the following 5 months (with the placebo mixture being identical to the intervention). After 6 months of treatment, the ADOS-CSS did not obtain a statistically significant difference. However, in an exploratory analysis, the NGI group treated with probiotics showed a significant reduction (p=0.026) in the total ADOS-CSS (which decreased from 6.72–5.91 in the probiotic group and increased from 6.96–7.17 in the placebo group). In addition, GI patients presented a statistically significant reduction in GI symptoms (6-GSI [p=0.009]), mainly in smell in feces and flatulence (p≤0.001), including a higher proportion of children with the normalization of sensory profile scores in the Multisensory Processing subscale (p=0.013).

Table 1 shows the statistical results and Table 2 shows the results found in the study.

## DISCUSSION

Probiotics have recently been used in several clinical trials as an additional treatment combined with conventional therapy in patients with ASD. This is due to the fact of the difference in the colonization of its microbiota when compared to that of neurotypical patients[8]. Children with ASD and GI symptoms have shown high levels of intestinal inflammation associated with dysbiosis[5,6,18] The probiotic approach should act as a tool to restore the healthy microbiota, in addition to reducing intestinal permeability and making negative regulation of inflammatory cytokines[19].

Regarding the analyzed studies, it is possible to observe heterogeneity regarding the use of probiotics (i.e., one study used a mixture and the other an isolated strain), and the dose used was variable, as well as the follow-up time. The studies were conducted in two different countries, which may lead to a broader evaluation, being a positive point observed.

The limitations of the studies include the reduced number of articles analyzed and the follow-up time, which limits the evaluation until the short term. The levels of markers of intestinal inflammation, as well as the evaluation of the intestinal microbiota made before and after the intervention with probiotic, did not present significant values in one study and was not performed in another, so there was no direct demonstration of its effects, focusing only on questionnaires performed.

The studies included in this article[16,17] demonstrated empirical improvements reported by parents and patients; however, there was less statistical significance in the reduction of total scores in the main questionnaires evaluated (i.e., CGI-I, ABC-T, ADOS-CSS, PedQL, and PRAS-ASD), with the exception

Table 2. Behavioral assessment by questionnaires and evaluation of symptom severity in patients with gastrointestinal symptoms.

| Study | Parameter | PR $T_0$ | PR $T_1$ | PL $T_0$ | PL $T_1$ | p-value† |
|---|---|---|---|---|---|---|
| Liu et al.[16] | ABC-T | 15.81 (8.39) | 14.67 (8.97) | 17 (9.31) | 16.21 (10.11) | 0.53 |
| | SRS | 138.87 (24.19) | 132.77 (22.99) | 135.88 (26.04) | 135.79 (25.79) | 0.63 |
| | CBCL | 49.63 (25.4) | 44.34 (23.25) | 50.60 (25.91) | 49.20 (24.46) | 0.53 |
| | SNAP-IV | 34.03 (14.61) | 31.87 (14.26) | 34.48 (13.39) | 33.16 (15.58) | 0.73 |
| Santocchi et al.[17] | ADOS-CSS | 6.84 (1.39) | 6.19 (1.56) | 6.97 (1.91) | 7.00 (1.80) | NS |
| | SCQ | 12.83 (6.68) | 11.97 (6.71) | 16.06 (5.54) | 13.90 (6.19) | NS |
| | RBS-R | 18.32 (13.17) | 14.37 (8.01) | 22.31 (15.47) | 19.13 (12.10) | NS |
| | DQ | 65.91 (18.06) | 69.27 (20.09) | 62.29 (20.12) | 61.14 (20.13) | NS |
| | VABS II | 63.87 (22.12) | 67.39 (22.29) | 57.00 (16.74) | 59.72 (16.38) | NS |
| | CBCL | 60.94 (9.94) | 57.80 (7.92) | 62.84 (10.97) | 57.30 (9.05) | NS |
| | PSI | 70.03 (29.63) | 66.62 (31.15) | 74.76 (24.98) | 61.03 (32.58) | NS |
| Santocchi et al.[17] | GSI, Smell of feces | 1.88 (0.33) | 0.56 (0.88) | 0.25 (0.71) | 0.14 (0.38) | <0.001 |
| | GSI, Flatulence | 0.56 (0.88) | 0.33 (0.50) | 0.43 (0.79) | 0.86 (0.99) | 0.0187 |
| | GSI, Total | 7.22 (1.99) | 2.89 (2.31) | 5.75 (1.03) | 3.43 (1.81) | 0.0416 |
| | 6-GSI, Total | 5.00 (1.22) | 1.67 (1.66) | 3.50 (0.93) | 2.00 (1.53) | 0.0191 |

†: p-value among the groups at the end of the intervention. Results expressed in standard deviation (SD). PR: probiotic; PL: placebo; NS: not significant; $T_0$: zero time; $T_1$: final time; ABC-T: autism behavior checklist-Taiwan; SRS: Social responsiveness scale; CBCL: Child behavior checklist; PSI: Parental stress index; SNAP-IV: Study version of the Swanson, Nolan, and Pelham, Version IV Scale; ADOS-CSS: Autism diagnostic observation schedule calibrated severity score; SCQ: Social communication questionnaire; RBS-R: Repetitive behavior scale-revised; VABS-II: Vineland adaptive behavior scales-second edition; GSI: Gastrointestinal severity index.

of SNAP-IV, which obtained a significant reduction (p=0.02) in the total score[17], especially in the group of younger patients (7–12 years) (p=0.004)[16]. They also showed better responses in the symptoms of inattention, hyperactivity/impulsivity, opposition/challenge, and rule-breaking behaviors[17], suggesting that younger patients may respond better to probiotic therapy in terms of behavioral aspects.

Santocchi et al.[17] divided patients with and without GI symptoms, obtaining different results among them, because only the NGI group, treated with probiotics, showed a significant reduction in ADOS-CSS (Total and Affective-Social scores), while patients in the GI group who received probiotics obtained improvement only in GI symptoms, with emphasis on reducing flatulence and smell in feces. These results corroborate other articles[20-22] that indicate the positive effect on the use of probiotics in patients with ASD, to improve GI symptoms such as constipation, abdominal pain, reduction of diarrhea, and improvement of stool consistency. Santocchi et al.[17] also indicated that the disparity between the results obtained by them can be explained by the heterogeneity in the composition of the microbiota of the participants, causing there to be potentially different effects on different targets.

In view of these results, the approach with probiotics showed low efficacy in improving behavioral symptoms, with some favorable outcomes[18] in patients with GI complaints, which could justify its use in complementary therapies. However, larger studies, with the laboratory microbiota analysis for better direction, should be conducted to attest or not to the efficacy of probiotic therapy in pediatric patients with ASD.

## AUTHORS' CONTRIBUTIONS

**MAA:** Conceptualization, Formal analysis, Methodology, Project administration, Supervision, Writing – original draft, Writing – review & editing. **MJRS:** Formal analysis, Supervision, Writing – original draft, Writing – review & editing. **ID:** Investigation, Methodology, Project administration, Writing – original draft. **JCC:** Investigation, Methodology, Project administration, Writing – original draft. **TCCS:** Investigation, Methodology, Project administration, Writing – original draft. **YAA:** Investigation, Methodology, Project Administration, Writing – original draft. **MRMO:** Validation, Resources, Writing – original draft, Writing – review & editing. **VEVR:** Supervision, Validation, Writing- review & editing.

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REVIEW ARTICLE
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# A review of genetic syndromes associated with hypertrichosis

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## INTRODUCTION

Hypertrichosis can be very troublesome for the affected patients and their families. This condition is characterized by an increase in hair growth beyond normal variation in areas that are not predominantly androgen-dependent, independent of age, race, or sex[1,2]. Hypertrichosis is classified according to the age of onset (congenital or acquired), the extent of distribution (generalized or localized), and whether it is isolated or associated with various abnormalities[2,3]. Further classification takes into consideration the type of follicle: lanugo, vellus, or terminal hair. Lanugo follicles are responsible for the growth of the first hairs, which are thin, soft, slightly pigmented, and nonmedullated, produced in the uterus, and are eliminated after birth. Lanugo hypertrichosis has been observed in adults with various forms of hypertrichosis. Vellus follicles are not medullated, thin, and poorly pigmented, and terminal hair is pigmented, medullated, and has a larger diameter compared with other types of hair[1,4].

The incidence of isolated hypertrichosis is unknown, and it is considered very rare. The incidence increases when it presents itself as a phenotype of several genetic syndromes[2]. Several causes of hypertrichosis have been described, including the use of drugs, infection, neoplasia, genetic diseases, and metabolic or nonendocrine disorders, but it is not caused by an excess of androgens[5]. This condition is often confused with hirsutism; however, the latter refers specifically to the growth of terminal hair in women or children, in androgen-dependent areas, and in places where there is normally no terminal hair, with a typical adult male distribution pattern[6].

There are several theories for the pathogenesis of hypertrichosis. First, it has been proposed to be caused by the conversion of intermediate or vellus hair to terminal hair, or from changes in the hair growth cycles, with follicles spending more time in the anagen phase and an increase in follicular density[1]. However, the triggers of these mechanisms are still not fully understood.

Hypertrichosis is not only a cutaneous sign but also an underlying rare complex disease that can affect multiple organ systems[1-3,7] and has previously been related to abnormalities in the head and neck, skeletal, nervous system, intellectual disability (ID), neoplasia, abdominal, genitourinary, cardiovascular, among others. However, there are only a few reviews in the literature. The aim of this study was to offer an overall survey of hypertrichosis-associated genetic diseases described in the literature and provide a summary of its clinical presentation.

## METHODS

A search was performed from June 2020 to October 2020 in the online electronic database *Online Mendelian Inheritance in Man* (OMIM, https://www.omim.org), with associations of the terms "hypertrichosis" or "hirsutism." Nondependent disturbances to androgen metabolism or syndromes with overlapping features were included as hypertrichosis. Additional searches were performed in the electronic databases PubMed (https://pubmed.ncbi.nlm.nih.gov) and Orphanet (https://www.orpha.net/consor/cgi-bin/index.php) to complement the search for scientific articles, in the English language.

The clinical features of each disturbance were organized into categories by one collaborator, as provided in OMIM, and

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features of the head and neck, inheritance, skeletal, cardiovascular, ID, nervous system, neoplasia, genitourinary, abdominal, endocrine, respiratory, dental anomalies, and phenotypic and genetic characteristics were also evaluated. The data were entered into Excel for statistical analyses. The study collected public domain data, thus dispensing with the approval of the Ethics and Research Committee.

## RESULTS

A total of 274 entries were found in OMIM. In 33 entries, both terms hypertrichosis and hirsutism were referring to the same disturbance. Notably, 121 genetic conditions associated with hypertrichosis were included in the research, as described in Chart 1. Description of genes and disturbances caused by hyperandrogenism or related conditions, such as polycystic ovarian syndrome, hyperprolactinemia, hyperthyroidism, congenital adrenal hyperplasia, androgen-secreting tumors, among others, were excluded. However, more than one OMIM entry can refer to the same syndrome. Disturbances with overlapping syndromes were also included. A few disturbances were not found in OMIM, but were found in PubMed (i.e., dysraphism, nevoid hypertrichosis, polythelia pilosa, primary multifocal hypertrichosis, and segmental odontomaxillary dysplasia). The distribution of the frequency of clinical involvement categories is described in Table 1.

The main inheritance pattern observed was autosomal recessive (44.62%). Nevertheless, some disturbances can occur with a mixed pattern. Autosomal dominance was observed in 36.36%, and other or unknown inheritance patterns were observed in 20.66% of genetic entities. The most affected categories observed were the head and neck features (80.16%), skeletal (78.51%), and the nervous system (73.55%).

Other highlighted categories were ID (52.06%), abdomen (42.97%), genitourinary (39.66%), dental anomalies (32.23%), cardiovascular (32.23%), respiratory (25.61%), and early death, until childhood (18.18%). Malignancies were of another concern, observed in 8.26% of cases, as described in Table 2, and endocrinopathies were identified in 14.04% of disturbances.

## DISCUSSION

There has been a growing recognition that rare diseases are relevant medical and social problems[8-10]. In this study, 121 genetic disturbances associated with hypertrichosis were identified. The first documented case of hypertrichosis in the scientific literature was the case of Petruz Gonzales, born in the Canary Islands archipelago in 1556, at the Ambras Castle[2]. Other cases later became famous, including those of circus exhibitionists, such as the case of Julia Pastrana, a Mexican dancer of indigenous origin, and the Russian Theodoro Petrov[11,12]. Although more than 300 new Mendelian phenotypes are added to the OMIM each year[13], only a few cases of hypertrichosis-associated genetic disturbances have been reported.

The prevalence of congenital generalized hypertrichosis is very rare[2]. Nevertheless, no universally accepted definition for rare diseases has yet been established[10,14]. According to the World Health Organization (WHO) and the criterion adopted by the Ministry of Health of Brazil, a rare disease is a disease whose prevalence affects less than 65/100,000 individuals or 1.3/2,000 individuals[15,16]. All conditions described in this study are rare.

Hypertrichosis can be classified as being associated with other symptoms, or as an isolated feature, but there are only a few examples of hypertrichosis as a cardinal symptom[17]. The majority of diseases express hypertrichosis as a component of complex syndromes[18], as shown in this study. Another classification is based on the localization hypertrichosis; however, the literature is not always clear enough to discern between localized and generalized hypertrichosis.

Head and neck features were the most affected category, identified in more than two-thirds of the disturbances; this includes abnormalities in the head, face, ears, eyes, nose, mouth, neck, and teeth, which reveal the importance of a thorough physical exam. Teeth abnormalities were identified in 32.23% of genetic entities. Dental anomalies are excellent dysmorphic markers and may help in syndrome diagnosis[12,19].

Skeletal involvement was identified in 78.51% of disturbances. Genetic skeletal disorders account for most human skeletal dysplasia; however, the genotype–phenotype correlations remain an important challenge[20]. Mutations in the same gene may be associated with heterogeneous phenotypes, as the same phenotype can be caused by mutations in several genes, such as Coffin–Siris, which has a wide genetic heterogeneity[20].

The nervous system was affected in 73.55% of the genetic entities. ID is a prominent feature observed in 52.06% of cases, usually identified early in childhood, due to developmental delay[7]. Given the greater clinical severity of the disease, its incidence is much higher than the worldwide prevalence, estimated at 1% of the general population[21]. ID is diagnosed by IQ testing; however, its severity (i.e., mild, moderate, severe, and profound) can be highly variable, even in the same disorder, given the wide heterogeneous phenotype of genetic diseases[21].

Another major concern is the association between hypertrichosis and cancer development, observed in 8.26% of cases (Table 2). In this context, different genes are associated, the main inheritance pattern observed is autosomal dominant, and the prognosis is usually poor. No correlation was found between the genetic entity and a unique type of malignancy, as

**Chart 1.** Genetic syndromes associated with hypertrichosis.

| Syndromes | |
|---|---|
| Achalasia–Microcephaly | Dental Anomalies And Short Stature |
| Adducted Thumbs Syndrome | Desanto–Shinawi Syndrome |
| Agenesis of corpus callosum, cardiac, ocular, and genital syndrome | Developmental and Epileptic Encephalopathy 57 |
| Alazami–Yuan Syndrome | Developmental and Epileptic Encephalopathy 85 With or Without Midline Brain Defects |
| Amaurosis Congenita, Cone–Rod Type, With Congenital Hypertrichosis | Diabetes Mellitus, Insulin Resistant, With Acanthoses Nigricans Type A |
| Anemia, Congenital Hypoplastic, With Multiple Congenital Anomalies/Mental Retardation Syndrome | Diarrhea, chronic, with villous atrophy |
| Barber–Say Syndrome | Distichiais, Tristichiasis |
| Becker Nevus Syndrome | Donohue Syndrome |
| Beckwith–Wiedemann Syndrome | Dysraphism |
| Bloom Syndrome | Dyssegmental Dysplasia, Rolland–Desbuquois Type |
| Bohring–Opitz Syndrome | Ectodermal Dysplasia 14, Hair/Tooth Type with or Without Hypohidrosis |
| Cahmr Syndrome | Ehlers–Danlos Syndrome, Dermatosparaxis Type |
| Cantu Syndrome | Erythroderma, Ichthyosiform, Congenital, Reticular |
| Cerebellar Ataxia, Mental Retardation, And Dysequilibrium Syndrome 2 | Erythrokeratodermia Variabilis Et Progressiva 2 |
| Cerebellar, Ocular, Craniofacial, And Genital Syndrome | Facial Dysmorphism, Hypertrichosis, Epilepsy, Intellectual/ Developmental Delay, And Gingival Overgrowth Syndrome |
| Cerebral Malformation, Seizures, Hypertrichosis, And Overlapping Fingers | Facial Hypertrichosis |
| Cerebrooculofacioskeletal Syndrome 1 | Fibromatosis, Gingival, With Hypertrichosis And Mental Retardation |
| Cervical Hypertrichosis with Underlying Kyphoscoliosis | Filippi Syndrome |
| Cervical Hypertrichosis, Anterior Cervical | Floating–Harbor Syndrome |
| Cervical Hypertrichosis, Congenital Anterior Cervical, with Peripheral Sensory and Motor Neuropathy | Fontaine Progeroid Syndrome |
| Chromosome 17q12 Deletion Syndrome | Frontometaphyseal Dysplasia 1 e 2 |
| Chromosome 17q21.31 Duplication Syndrome | GM-1 – Gangliosidosis type I |
| Coffin–Siris Syndrome 1, 2, 3, 4, 8 ,9 | Hairy Ears; Hairy Ears, Y-Linked |
| Congenital Disorder Of Glycosylation Iaa, Iq e Ile | Hairy Elbows |
| Cornelia De Lange Syndrome 1, 3, 4 | Hairy Palms and Soles |
| Corpus Callosum, Agenesis Of, With Abnormal Genitalia | Hajdu–Cheney Syndrome |
| Cousin Syndrome | Hennekam Lymphangiectasia–Lymphedema Syndrome 1 |
| Craniorhiny | Histiocytosis–Lymphadenopathy Plus Syndrome H Syndrome, Rosai–Dorfman Disease, Familial |
| Crouzon Syndrome | Hydronephrosis Congenital, With Cleft Palate, Characteristic Facies, Hypotonia, Mental Retardation |
| Curry–Jones Syndrome | Hypertrichosis lanuginosa; congenital; with/without gingival hyperplasia; Ambras |

Continue...

Chart 1. Continuation.

| Syndromes | |
|---|---|
| Hypomelanosis of Ito | Neurodevelopmental Disorder With Progressive Microcephaly, Spasticity, And Brain Anomalies |
| Intellectual developmental disorder with cardiac defects and dysmorphic facies | Nevoid Hypertrichosis |
| Imagawa–Matsumoto Syndrome | Oliver–Mcfarlane Syndrome |
| Immunodeficiency 49 | Perching Syndrome |
| Joubert Syndrome 10 | Polythelia Pilosa |
| Kabuki Syndrome 2 | Pontocerebellar Hypoplasia Type 8 |
| Leigh Syndrome | Porphyria Cutanea Tarda I, Ii,Porphyria, Congenital Erythropoietic Variegate Porphyria |
| Lethal Short-Limb Skeletal Dysplasia, Al Gazali Type | Primary Multifocal Localized Hypertrichosis |
| Leukodystrophy, Hypomyelinating, 17 | Ramon Syndrome |
| Liang–Wang Syndrome | Rubinstein–Taybi Syndrome I, Ii |
| Lichtenstein Syndrome | Sandestig–Stefanova Syndrome |
| Light Fixation Seizure Syndrome | Schinzel–Giedion Midface Retraction Syndrome |
| Lipodystrophy, Congenital Generalized, Type 2 Berardinelli–Seip Syndrome | Schwartz–Jampel Syndrome, Type 1 |
| Lissencephaly 7 With Cerebellar Hypoplasia | Seckel Syndrome 9 |
| Lymphedema–Hypoparathyroidism Syndrome | Segmental Odontomaxillary Dysplasia |
| Mandibulofacial Dysostosis With Macroblepharon And Macrostomia | Sialuria |
| Mannosidosis, Alpha B, Lysosomal | Spastic Paraplegia 53, Autossomal Recessive |
| Marshall–Smith Syndrome | Specific Granule Deficiency 2 |
| Meester–Loeys syndrome | Spinocerebellar Ataxia 42, Early-Onset, Severe, With Neurodevelopmental Deficits |
| Melanocytic Nevus Syndrome | Spinocerebellar Ataxia, Autosomal Recessive 20 |
| Mental Retardation, Autosomal Dominant 57 | Spondyloepimetaphyseal Dysplasia, Genevieve Type |
| Mental Retardation, Autosomal Recessive 35 | Stocco Dos Santos X-Linked Mental Retardation Syndrome |
| Mental Retardation, Microcephaly, Epilepsy, And Coarse Face | Sweeney–Cox Syndrome |
| Mental Retardation, X-Linked 99, Syndromic, Female-Restricted | Tenorio Syndrome |
| Mental Retardation, X-Linked, Syndromic, Chudley–Schwartz Type | Trichohepatoneurodevelopmental Syndrome |
| Mental Retardation, X-Linked, Syndromic, Nascimento Type | Trichomegaly |
| Michelin Tire Baby Syndrome | Vissers–Bodmer Syndrome |
| Mitochondrial Complex I Deficiency, Nuclear Type 23 | Warburg Micro Syndrome |
| Mucopolysaccharidosis, Type Ii, Iiic, Iiid, Vii | Wiedemann–Steiner Syndrome |
| Mullerian Derivatives, Persistence Of, With Lymphangiectasia And Postaxial Polydactyly | Zimmermann–Laband Syndrome 1 |
| Multicentric Osteolysis, Nodulosis, And Arthropathy | |

one condition can be associated with several types of malignancies, but some may occur more often than others. For example, melanocytic nevus syndrome is associated with melanoma, and Beckwith–Wiedemann is associated with Wilms tumor and hepatoblastoma[22,23]. Nevertheless, Bloom syndrome and Schinzel–Giedion syndrome are associated with multiple malignancies[24,25]. In other genetic diseases, such as the Bohring–Opitz syndrome and Rubinstein–Taybi syndrome, tumor predisposition has been observed in many case reports, but the risk cannot be established or fully dismissed because epidemiologic studies have not been conducted to demonstrate an increased risk of developing cancer[26,27].

Hypertrichosis is not caused by androgens but is often confused with hirsutism, which is usually associated with hyperandrogenism. In this study, 17 conditions were associated with endocrinopathies. The most common abnormalities were diabetes mellitus, insulin resistance, and thyroid dysfunction (hypothyroidism and thyroid lymphangiectasis). Diabetes mellitus, insulin resistance, acanthosis nigricans type A, and Donohue syndrome are caused by a mutation in the insulin receptor gene (INSR) and are associated with insulin resistance and hyperinsulinemia[28,29]. Another example is the Berardinelli–Seip syndrome, which is associated with polycystic ovary disease, diabetes mellitus, and the Beckwith–Wiedemann syndrome, which is associated with adrenocortical cytomegaly and pituitary hyperplasia[23,30]. However, the Donohue syndrome, Berardinelli–Seip syndrome, and Beckwith–Wiedemann syndrome are the major causes of hypertrichosis in the literature[1,2,18,23]. One probable reason why these genetic conditions are classified as

Table 1. Clinical features of hypertrichosis associated genetic syndromes.

| | Syndromes n (%) |
|---|---|
| Head and neck | 97 (80.16) |
| Skeletal | 95 (78.51) |
| Nervous system | 89 (73.55) |
| Intellectual disability | 63 (52.06) |
| Autosomal recessive | 54 (44.62) |
| Abdominal | 52 (42.97) |
| Genitourinary | 48 (39.66) |
| Autosomal dominant | 44 (36.36) |
| Cardiovascular | 39 (32.23) |
| Dental anomalies | 39 (32.23) |
| Respiratory | 31 (25.61) |
| Other or unknown inheritance pattern* | 25 (20.66) |
| Early death (until childhood) | 22 (18.18) |
| Endocrine | 17 (14.04) |
| Neoplasia | 10 (8.26) |

*X-linked, Y-linked, somatic mosaicism, somatic mutation, and isolated cases.

Table 2. Genetic disturbances with hypertrichosis associated with neoplasia.

| Syndrome | OMIM | Inheritance | Gene | Chromosome |
|---|---|---|---|---|
| Beckwith–Wiedemann | 130650 | AD | H19; ICR1; KCNQ10T1; CDKN1C | 11p15.5 11p15.4 |
| Bloom | 210900 | AR | RECQL3 | 15q26.1 |
| Bohring–Opitz | 605039 | AD | ASXL1 | 20q11.21 |
| Curry–Jones | 611707 | Somatic mosaicism | SMO | 7q32.1 |
| Donohue | 246200 | AR | INSR | 19p13.2 |
| Melanocytic nevus | 137550 | Somatic mutation | NRAS | 1p13.2 |
| Polythelia pilosa | – | – | – | – |
| Porphyria<br>Cutanea tarda I, II<br>Congenital erythropoietic porphyria<br>Variegate porphyria | 176090<br>176100<br>263700<br>176200 | AD–AR<br>AR<br>AD | UROD<br>UROS<br>PPOX | 1p34.1<br>10q26.2<br>1q23.3 |
| Rubinstein–Taybi I, II | 180849<br>613684 | AD<br>AD | CREBBP<br>EP300 | 16p13.3<br>22q13.2 |
| Schinzel–Giedion midface retraction syndrome | 269150 | AD | SETBP1 | 18q12.3 |

AD: autosomal dominant; AR: autosomal recessive; OMIM: Online Mendelian Inheritance in Man.

hypertrichosis is that hyperandrogenism may aggravate the problem, as hypertrichosis has also been described in adult males, not only in androgen-dependent areas[1,2,18].

Hypertrichosis can cause significant emotional distress for affected patients and their families[1,18]. Patients may experience difficulty in accessing the qualified health system, as the clinical characteristics are heterogeneous and can lead to diagnosis delays[15]. Early diagnosis of these conditions helps guide early intervention, screening, and genetic counseling of patients and their family members. The development of clinical protocols helps health professionals, patients, and families to make decisions regarding the most appropriate alternatives for their healthcare.

There is a limitation in the interpretation of data from case reports, with a small number of patients, an inherent characteristic of rare disease studies. The literature is not always clear enough to elucidate the type of hair disorder, whether hypertrichosis or hirsutism. In fact, it is common for both terms to be used in case reports of the same genetic disorder. It was imperative to deepen the knowledge to perform the necessary discernment to conduct the work and exclude what was not the object of investigation of the study.

## CONCLUSIONS

This study shows that hypertrichosis may be more common than estimated, especially when we consider it to be a phenotype of several diseases. The research also suggested that cutaneous manifestations may also hide an underlying disease that requires investigation. Multiple organ systems can be affected, and the study highlights the most affected ones. These aspects reinforce the need for further studies to support protocols for public organizations and policies, facilitate decision-making, and promote ongoing health training for the management of hypertrichosis and its underlying potential disorders.

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## AUTHORS' CONTRIBUTIONS

**VFC:** Conceptualization, Data curation, Formal analysis, Writing – original draft, Writing – review and editing. **MCB:** Conceptualization, Data curation, Formal analysis, Writing – original draft. **DRBM:** Conceptualization, Writing – review and editing. **MJBA:** Formal analysis, Writing – review and editing. **PRB:** Formal analysis, Writing – review and editing. **HMJ:** Conceptualization, Data curation, Formal analysis, Writing – review and editing.

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COMMENTARY
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# Comment on "Prevalence of hot flashes in women of 40 to 65 years of age with metabolic syndrome"

Rong Hu[1], Zhongguo Ren[2]*

Dear Editor,

We were very pleased to read the article "Prevalence of hot flashes in women of 40–65 years of age with metabolic syndrome" by Sau and colleagues[1]. In this study, the authors revealed that hot flashes are highly prevalent among women older than 40 years and appear to be associated with metabolic syndrome (MetS). The occurrence of hot flashes may be a risk marker for MetS. However, some concerns should be raised from our point of view.

First, this was a cross-sectional study based on information obtained from 7212 women who were followed up in 2014 via the Family Health Program in the city of Pindamonhangaba (Brazil). A potential for selection bias exists; out of 1435 women who were initially recruited, 513 women who did not complete the questionnaire and who were employed for the calculation of the Kupperman index were excluded from the study. Our suggestion is that all 7212 women who were followed up in 2014 should be included in this study.

Second, the authors mentioned that multiple logistic regression models were constructed to estimate the relative weight of each independent variable after adjustment for skin color, income, depression, anxiety, and smoking in the Statistical calculations section. However, we did not find any analysis on skin color, income, depression, anxiety, and smoking status among the women included. Additionally, the scale for evaluation of anxiety and depression should be described in detail in the Methods section.

## AUTHORS' CONTRIBUTIONS

**RH:** Data curation, Formal Analysis, Writing – original draft.
**ZR:** Conceptualization, Writing – review and editing.

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1. Sau HPF, Schmitt ACB, Cardoso MRA, Aldrighi JM. Prevalence of hot flashes in women of 40 to 65 years of age with metabolic syndrome. Rev Assoc Med Bras. 2020;66(12):1628-32. https://doi.org/10.1590/1806-9282.66.12.1628

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COMMENTARY
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# Comment on "Expression of long noncoding RNA NBAT1 is associated with the outcome of patients with non-small cell lung cancer"

Jing Ci[1], Li Liu[2], Xin Wang[3]*

Dear Editor,

We read the article "Expression of long noncoding RNA NBAT1 is associated with the outcome of patients with non-small cell lung cancer" by Wang et al.[1] with great interest. Although long noncoding RNA neuroblastoma-associated transcript one expression might contribute to tumor progression and poor prognosis of non-small cell lung cancer (NSCLC), some points should be raised from our point of view.

First, what is the basis for grouping the expression of long noncoding RNA NBAT1 into two groups. For example, the cutoff value is the average NBAT1 expression level of all patients. As shown in Figure 1, expression of long noncoding RNA NBAT1 was not normally distributed. Thus, the median value of the NBAT1 expression level should be chosen as the cutoff criterion to dichotomize into high- and low-expression subgroups.

Second, more *in vitro* studies should be done to confirm this finding. The study found that low NBAT1 level was also significantly associated with lymph node metastasis.

It is known that lymph node metastasis was associated with poor prognosis of NSCLC. The reason for these apparently contradictory results is not clear.

## AUTHORS' CONTRIBUTIONS

**JCH:** Data curation, Formal analysis, Writing – original draft.
**LL:** Data curation, Formal analysis, Writing – original draft.
**XW:** Conceptualization, Writing – review and editing.

## REFERENCE

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COMMENTARY
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# Comment on "Efficacy and safety of percutaneous transforaminal endoscopic discectomy in the treatment of lumbar spinal stenosis combined with osteoporosis"

Jie Ding[1]*, Qiong-mei Han[2]

Dear Editor,

We read the article "Efficacy and safety of percutaneous transforaminal endoscopic discectomy in the treatment of lumbar spinal stenosis combined with osteoporosis" by Gu et al.[1] with a great interest. The study found that percutaneous transforaminal endoscopic discectomy (PTED) is safe and effective in the treatment of lumbar spinal stenosis (LSS) combined with osteoporosis. However, some issues should be addressed from our point of view.

First, no published references were found for the evaluation of treatment efficacy. It is not a reasonable grouping for weekly exercise time. In the Results section, the study found that compared with the control group, the operation time, blood loss, and hospitalization duration in the PTED group were significantly decreased ($p<0.05$). The above sentence is a statistical misrepresentation. It should be stated that statistically significant difference was found for the operation time, blood loss, and hospitalization duration between two groups.

Second, another concern is the application of incorrect statistical methods in this study, because the treatment variable to assess the treatment effect is an ordered categorical data. The most appropriate statistical analysis methods for ordered categorical data should be nonparametric tests, for example, Mann-Whitney rank-sum test. However, the chi-square test was used to compare the treatment effects of two groups in this study.

## AUTHORS' CONTRIBUTIONS

**JD:** Data curation, Formal analysis, Writing – original draft.
**QMH:** Conceptualization, Writing – review and editing.

## REFERENCE

1. Gu X, Zhu W, He H, Wang Z, Ding S, Guo G. Efficacy and safety of percutaneous transforaminal endoscopic discectomy in the treatment of lumbar spinal stenosis combined with osteoporosis. Rev Assoc Med Bras (1992). 2019;65(6):779-85. https://doi.org/10.1590/1806-9282.65.6.779

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COMMENTARY
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# Comment on "Roles of certain biochemical and hematological parameters in predicting mortality and ICU admission in COVID-19 patients"

Liwen Liu[1], Xiaofei Li[1]*

Dear Editor,

We were very pleased to read the article "Roles of certain biochemical and hematological parameters in predicting mortality and ICU admission in COVID-19 patients" by Bilgir and colleagues[1]. In the study, the authors revealed that the most effective parameters to predict intensive care unit admission and mortality in COVID-19 patients are ferritin, lactate dehydrogenase, D-dimer, C-reactive protein, red cell distribution width, and creatinine. This study provides very valuable insight for preventing the COVID-19 pandemic. However, some concerns should be raised from our point of view.

First, there is an apparent age difference in this study. The mean age was 56.0±18.5 years, where the youngest was 18 and the oldest was 96 years old. Thus, age-stratified analysis could reduce the impact of age on the results. In contrast, gender of patients also affected intensive care unit admission and mortality in the COVID-19 patients.

Second, several statistical tests are described separately under the Statistical analysis section in Methods. However, the specific statistical analysis cannot be found, for instance, use of the Pearson's $\chi^2$ or Fisher's exact $\chi^2$ test in the Results section and corresponding figure legends. To further explore the potential risk factors for mortality, multivariable Cox regression analysis with backward-stepwise selection could be used to identify the predictors of mortality in the COVID-19 patients.

## AUTHORS' CONTRIBUTIONS

**LL:** Data curation, Formal Analysis, Writing – original draft.
**XL:** Conceptualization, Writing – review & editing.

## REFERENCE

1. Bilgir F, Çalık Ş, Demir I, Bilgir O. Roles of certain biochemical and hematological parameters in predicting mortality and ICU admission in COVID-19 patients. Rev Assoc Med Bras (1992). 2021;67(Suppl 1):67-73. https://doi.org/10.1590/1806-9282.67.Suppl1.20200788

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COMMENTARY
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# Comment on "Smoking prevalence and effects on treatment outcomes in patients with tuberculosis"

Liwen Liu[1], Xiaofei Li[1]*

Dear Editor,

We were very pleased to read the article "Smoking prevalence and effects on treatment outcomes in patients with tuberculosis" by Vargas and colleagues[1]. In this study, the authors revealed that active smokers had less chance for cure and more abandonment than non-active smokers. This study provides very valuable insight for tuberculosis (TB) control, especially in cognitive–behavioral approaches to smoking cessation. However, some concerns should be raised from our point of view.

First, though smoking status was determined according to the definitions of the Centers for Disease Control and Prevention, a brief description of smoking status should also be provided in the Materials and methods section. There is an apparent sex difference among active smoking patients involved in this study. Thus, sex-stratified analysis could reduce the impact of sex on the results. These results were influenced not only by the subjects' gender but also by smoking history.

Second, the reliability and validity of the Fagerström scale have not been assessed in this study. Also, the statistical values should be provided. Further studies are needed to determine more sociodemographic characteristics, including age, occupation, education level, ethnicity, parity, and residency. It may be found that more vital risk factors also play an important role in the control of TB.

## AUTHORS' CONTRIBUTIONS

**LL:** Data curation, Formal Analysis, Writing – original draft.
**XL:** Conceptualization, Writing – review and editing.

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1. Vargas KR, Freitas AA, Azeredo ACV, Silva DR. Smoking prevalence and effects on treatment outcomes in patients with tuberculosis. Rev Assoc Med Bras (1992). 2021;67(3):406-10. https://doi.org/10.1590/1806-9282.20200825

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